

## Supporting Information

### **Two wavelength-shifting molecular beacons for simultaneous and selective detection of vesicular miRNA-21 and miRNA-31 in living cancer cells**

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## 2. Materials and methods

Chemicals and dry solvents were purchased from *Aldrich*, *ABCR* and *VWR* were used without further purification unless otherwise mentioned. Unmodified DNA strands were obtained from *Metabion*. TLC was performed on ALUGRAM Sil G/UV<sub>254</sub> 0.20 nm silica gel 60 F254 from *Macherey-Nagel GmbH & Co. KG*. Flash chromatography was carried out with silica gel 60 from *Aldrich* (60 – 43 μm). Spectroscopic measurements were recorded in NaP<sub>i</sub> buffer solution (10 mM, pH = 7) in presence of 250 mM NaCl (see detailed description for each experiment) using quartz glass cuvettes (10 mm). Absorption spectra were recorded with a *Varian Cary 100* spectrometer equipped with a 6x6 cell changer unit at 20 °C. Fluorescence was measured with a *Jobin–Yvon Fluoromax 3* fluorimeter with a step width of 1 nm and an integration time of 0.2 s. All spectra were recorded at 20 °C and are corrected for Raman emission from the buffer solution. NMR spectra were recorded on a *Bruker B-ACS-60*, *Bruker Avance DRX 400* and a *Bruker Avance DRX 500* spectrometer in deuterated solvents (<sup>1</sup>H at 300, 400 or 500 MHz, <sup>13</sup>C at 75, 100 or 125 MHz). Chemical shifts are given in ppm relative to TMS. IR spectra recording were performed by the Institute of Organic Chemistry of the KIT with a *Bruker IFS88*. DNA strands were purified with a Reversed Phase *Supelcosil™* LC-C18 column (250 x 10 mm, 5 μm) on a *Shimadzu* HPLC system (autosampler SIL-10AD, pump LC-10AT, controller SCL-10A, diode array detector SPD-M10A). Purification was verified by MS (MALDI) on a *Biflex-IV* spectrometer from *Bruker Daltonics* in the linear negative mode and *Autoflex-III Smartbeam* from *Bruker Daltonics* in the linear negative mode (matrix for DNA: 2:1 mixture of 2,4,6-trihydroxyacetophenone (0.3 M in ethanol) and diammoniumcitrate (0.1 M in H<sub>2</sub>O)). Finally, the oligonucleotides were lyophilized and quantified by their absorbance (in water at 260 nm on a ND-1000 spectrophotometer from *NanoDrop* in the nucleic acid mode). Quantum yields were determined with *Quantaaurus QY C11347* of *Hamamatsu*. Live cell imaging was performed on a *Leica TCS SP5 II* inverted confocal microscope (*Leica Microsystems GmbH, Wetzlar, Germany*) using a 63.0x1.40 OIL objective lens (37 °C, 5 % CO<sub>2</sub>).

### 3. Synthesis of the dyes X<sup>1</sup>, X<sup>2</sup>, Y<sup>1</sup>, Y<sup>2</sup> and their corresponding azides

Synthesis of the dyes X<sup>1</sup>, X<sup>2</sup>, Y<sup>2</sup> and their corresponding azides are already published.<sup>[1,2]</sup>

Synthesis of dye Y<sup>1</sup> and the corresponding azide is already published.<sup>[3]</sup>

## 4. Preparation and purification of DNA

### 4.1 Synthesis of DNA (MB1 and MB2)

Because of the lability of the cyanine-styryl dyes toward bases the solid-phase *ultramild-protecting* methodology was used for the synthesis of **MB1** and **MB2**.<sup>[4]</sup> The corresponding DNA building blocks (phosphoramidites) are commercially available and bear phenoxyacetyl, methoxyacetyl, and isobutyryl protecting groups at the DNA bases. Oligonucleotides (molecular beacons = MB) were prepared on an Expedite 8909 Synthesizer from *Applied Biosystems (ABI)*. Reagents and controlled pore glass (CPG) (1 μmol) were purchased from *ABI* and *Glen Research*. 2'-Propargyl-uridine (cU) and *ultramild base* phosphoramidites were purchased from *ChemGenes*. DNA synthesis was performed using standard coupling conditions since the cU building block contains a phosphoramidite group at the 3'-position and a DMT group at the 5'-position. The concentration of the artificial building block was 0.1 mol/L (in acetonitrile).

After incorporation of the first 2'-propargyl-modified uridine into the sequence, three more nucleosides were incorporated, the automated DNA synthesis was stopped and the first "Click"-reaction with the first dye azide was performed manually on solid phase (90 min at 60 °C). Using the washed (see 4.2) and carefully dried resin, the automated DNA synthesis was resumed. After completion, the second "Click"-reaction was performed, again manually and on solid phase.

## 4.2 “Click” modification of MB1 and MB2

For post-synthetic labeling the MBs with two different fluorophores at two specific sites of the oligonucleotides the “Click”-reaction was used. Therefore reagent solutions were prepared and the resin was soaked with a mixture of the following components:

- 50  $\mu\text{L}$  (20  $\mu\text{mol}$ ) of an aqueous sodium ascorbate solution (400 mM in  $\text{H}_2\text{O}$ )
- 130  $\mu\text{L}$  acetonitrile
- 228  $\mu\text{L}$  (2.28  $\mu\text{mol}$ ) of dye azide (10 mM in DMSO / t-butanol = 3 : 1)
- 34  $\mu\text{L}$  (3.4  $\mu\text{mol}$ ) of a solution of tetrakis(acetonitrile)copper(I)-hexafluorophosphate (100 mM DMSO / t-butanol = (3 / 1))

The reaction solution was kept 90 min at 60 °C and was mixed every 30 min to ensure complete reaction. After cooling to room temperature the mixture was removed and the solid support was washed subsequently with 3 mL of the following different solvents or solutions, respectively.

- Acetonitrile
- $\text{H}_2\text{O}$
- $\text{Na}_2\text{EDTA}$ -solution (approx.. 130 mg in 9 mL  $\text{H}_2\text{O}$ )
- $\text{H}_2\text{O}$
- Ethanol
- and finally acetonitrile (several times)

Then the solid support was dried and the automated DNA synthesis was resumed or the oligonucleotides were cleaved off the dried resin and deprotected using 1.0 mL of 0.05 mol/L  $\text{K}_2\text{CO}_3$  in methanol. The suspension was mixed 15 h at 25 °C. Approximately 6  $\mu\text{L}$  acetic acid were added to the separated methanol phase of the deprotection solution. The oligonucleotide was precipitated and centrifuged. The pellet was solubilized in 250  $\mu\text{L}$   $\text{H}_2\text{O}$  for HPLC-purification.

### 4.3 HPLC-purification and MALDI Mass spectra of modified DNA

The modified oligonucleotides were purified by HPLC Reversed Phase [*Supelcosil™* LC-C18 column (250 x 10 mm, 5  $\mu$ m)] on a *Shimadzu* HPLC system [autosampler SIL-10AD, pump LC-10AT, controller SCL-10A, diode array detector SPD-M10A]; using the following conditions:

A)  $\text{NH}_4\text{OAc}$  buffer (50 mM), pH = 6.5    B) Acetonitrile; for gradient see Table **S1**

flow rate = 2.5 mL/min

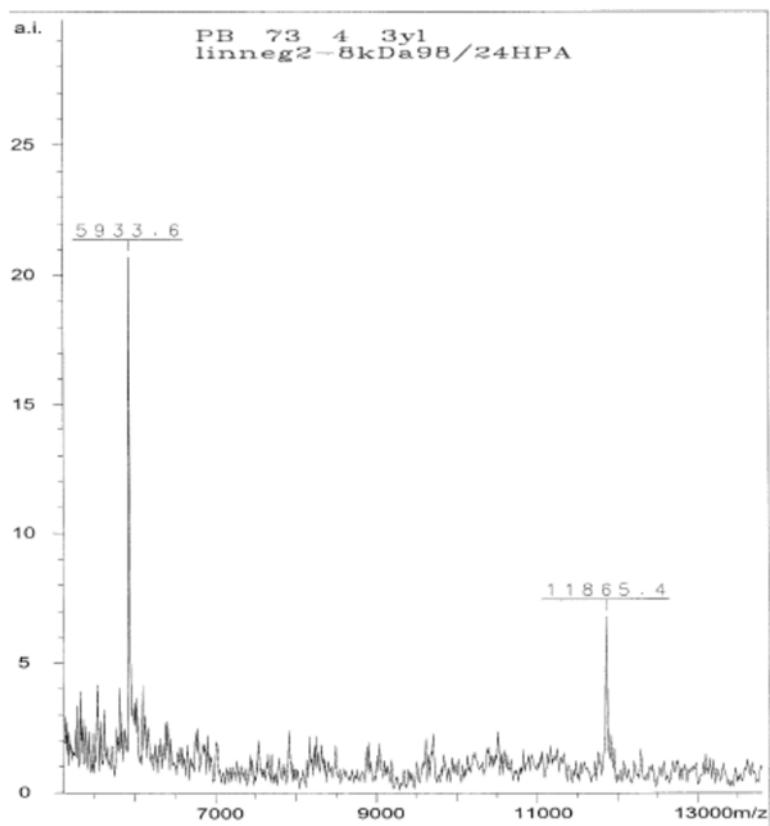
temperature = 40 °C

UV/Vis detection at 260, 459 and 542 nm for **MB1** and 260, 391 and 506 nm for **MB2**.

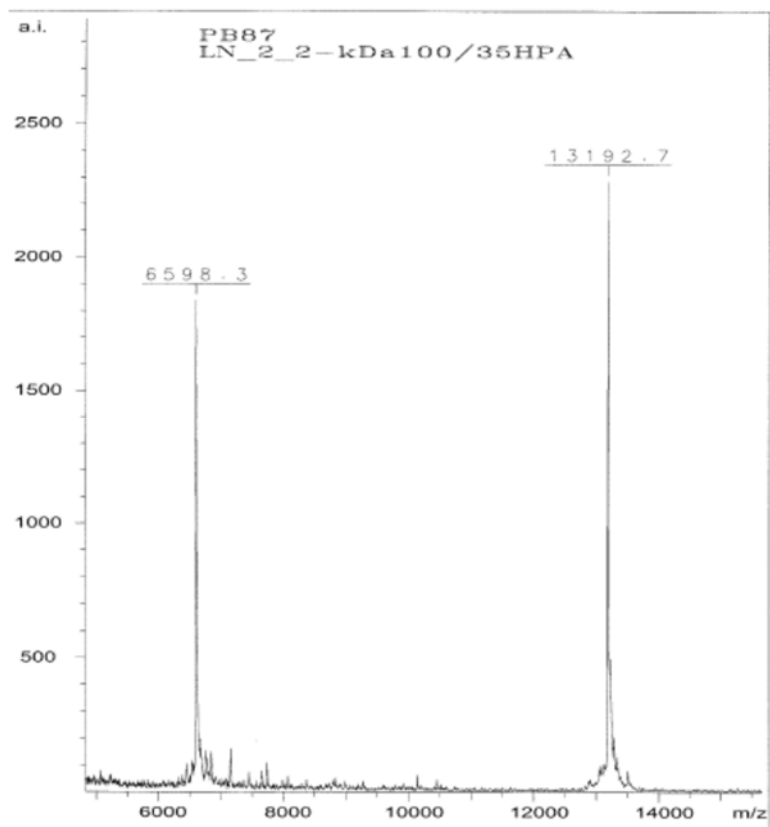
**Table S1.** HPLC-gradients for semi-preparative HPLC purification of **MB1** and **MB2**.

time [min]	amount of eluent B [%]	
	MB1	MB2
0	0	0
45	17	15
75	17	15
76	95	95
85	95	95
86	0	0
95	0	0

Purification was verified by MS (MALDI) on a Biflex-IV spectrometer from *Bruker Daltonics* in the linear negative mode and Autoflex-III Smartbeam from *Bruker Daltonics* in the linear negative mode (matrix for DNA: 2:1 mixture of 2,4,6-trihydroxyacetophenone (0.3 M in ethanol) and diammoniumcitrate (0.1 M in  $\text{H}_2\text{O}$ )).



**Figure S1.** MALDI spectra of **MB1**, calculated: 11859.3 g/mol; found: 11865.4 g/mol.



**Figure S2.** MALDI spectra of **MB2**, calculated: 13186.4 g/mol; found: 13192.7 g/mol.

## 5. Quantum yields

Quantum yields  $\phi_F$  were determined with 2.5  $\mu\text{M}$  MB, without and with 1.5 equivalents miRNA target in 10 mM  $\text{NaP}_i$ -buffer (10 mM, pH = 7) in presence of 250 mM NaCl using Quantaurus QY C11347 of Hamamatsu.

**Table S2.** Quantum yields of **MB1** and **MB2** without and with 1.5 equivalents miRNA-target, respectively.

Notation	MB (without miRNA)		with 1.5 eq miRNA-target	
	emission color	$\phi_F$ [%]	emission color	$\phi_F$ [%]
<b>MB1</b>	red	12.5	green	11.3
<b>MB2</b>	yellow	17.2	blue	1.5

## 6. Melting temperatures

$\lambda = 260$  nm; 10 - 90 °C; interval: 0.5 °C/min; 2.5  $\mu\text{M}$  MB, without and with 1.5 equivalents miRNA target in 10 mM  $\text{NaP}_i$ -buffer (pH = 7.0) and 250 mM NaCl.

**Table S3.** Melting temperatures of **MB1** and **MB2** without and with 1.5 equivalents miRNA-target, respectively.

Notation	MB (without miRNA)	with 1.5 eq miRNA-target
<b>MB1</b>	47.5 °C	73.6 °C
<b>MB2</b>	47.7 °C	76.9 °C

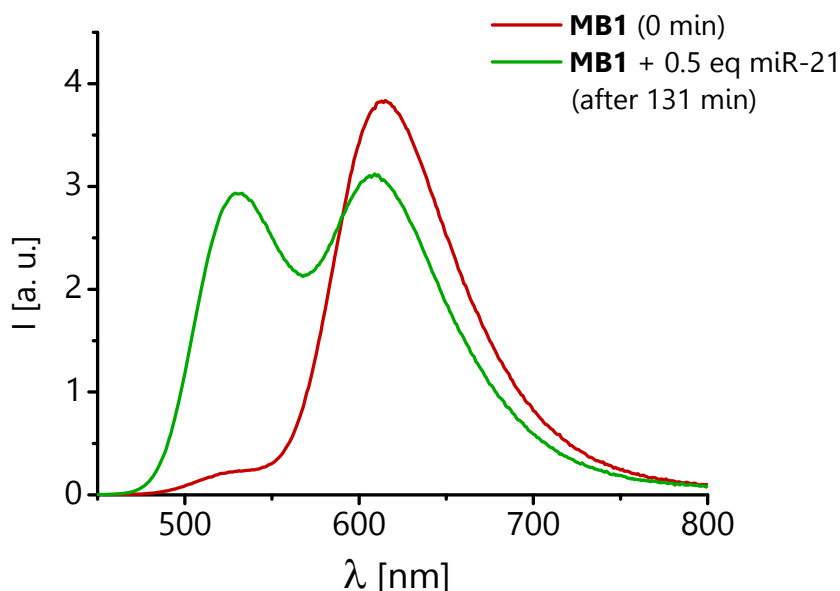


## 7. Spectroscopic data

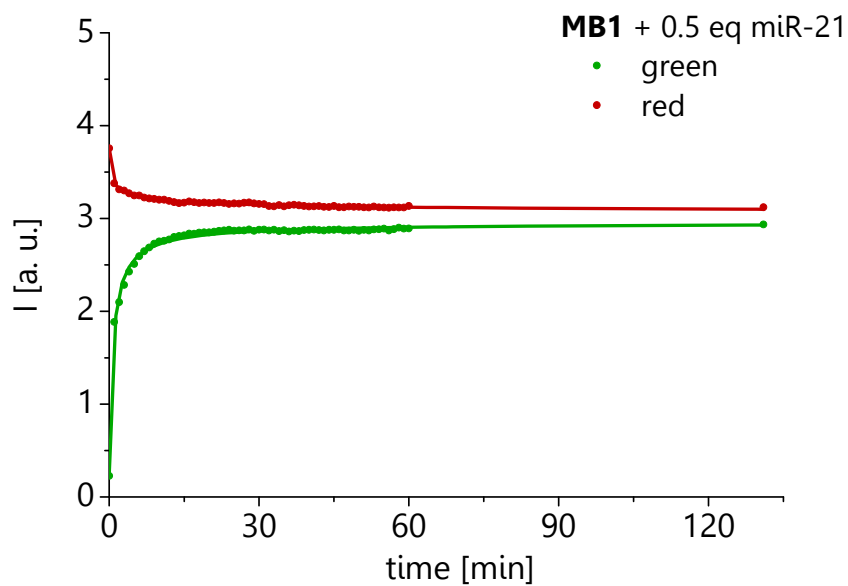
Spectroscopic measurements were recorded with 2.5  $\mu\text{M}$  DNA in  $\text{NaP}_i$  buffer solution (10 mM, pH = 7 and 250 mM NaCl) using quartz glass cuvettes (10 mm) at 20 °C. Absorption spectra were recorded with a *Varian Cary 100* spectrometer equipped with a 6x6 cell changer unit. Fluorescence was measured with a *Jobin–Yvon Fluoromax 3* fluorimeter with a step width of 1 nm and an integration time of 0.2 s. Spectra were recorded with an excitation band pass and an emission band pass of 3 nm and were corrected for Raman emission from the buffer solution (**MB1** with  $\lambda_{\text{exc.}} = 435$  nm;  $\lambda_{\text{em.}} = 450 - 800$  nm and **MB2** with  $\lambda_{\text{exc.}} = 391$  nm;  $\lambda_{\text{em.}} = 401 - 766$  nm).

Endpoint determination measurements were recorded after addition of 0.5 equivalents of miRNA-target and titration experiments up to an addition of 4.3 or 5.0 equivalents of miRNA-target, respectively to previously annealed **MB1** or **MB2**. Thereby, following the time to equilibrium determined in endpoint determination measurements.

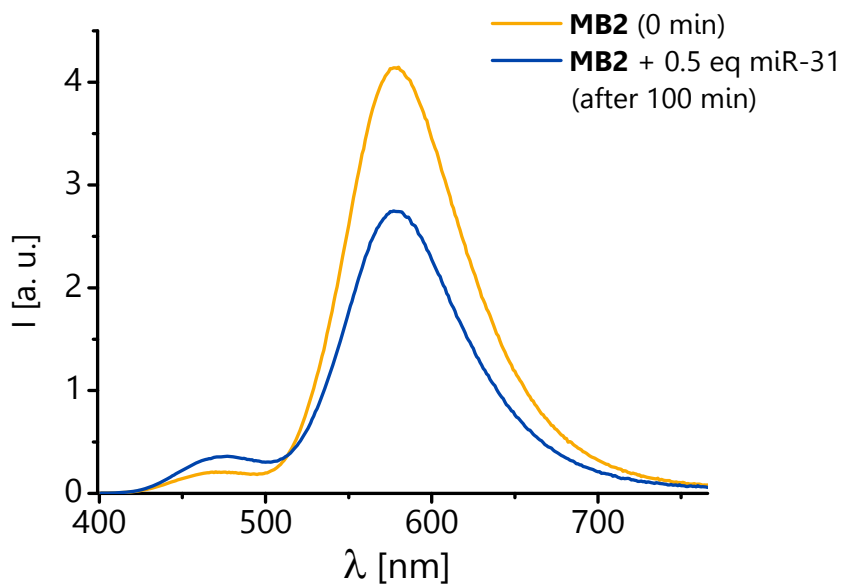
### 7.1 Spectroscopic data of endpoint determinations



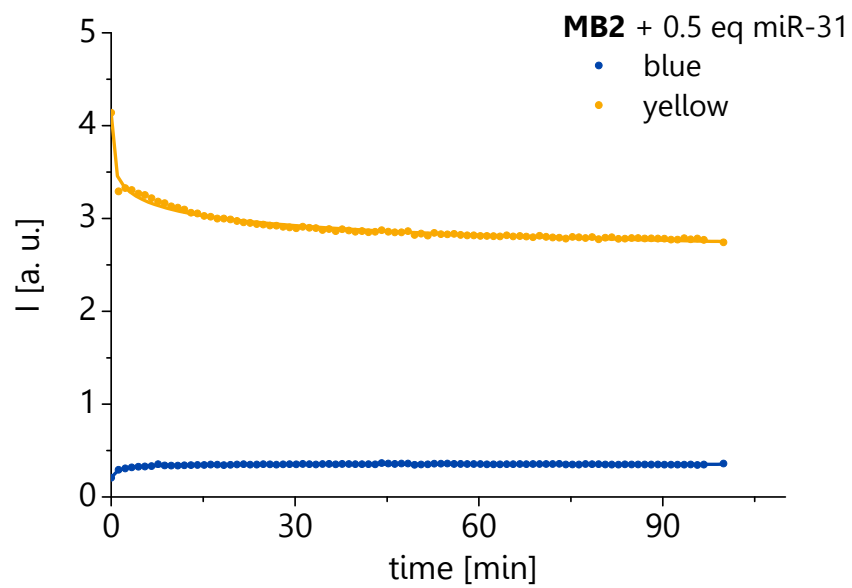
**Figure S3.** Fluorescence spectra after 0 min (start) and after 131 min (end) of endpoint determination of **MB1**.



**Figure S4.** Fluorescence intensity changes of dye X<sup>1</sup> (green) and dye Y<sup>1</sup> (red) of endpoint determination of **MB1**.

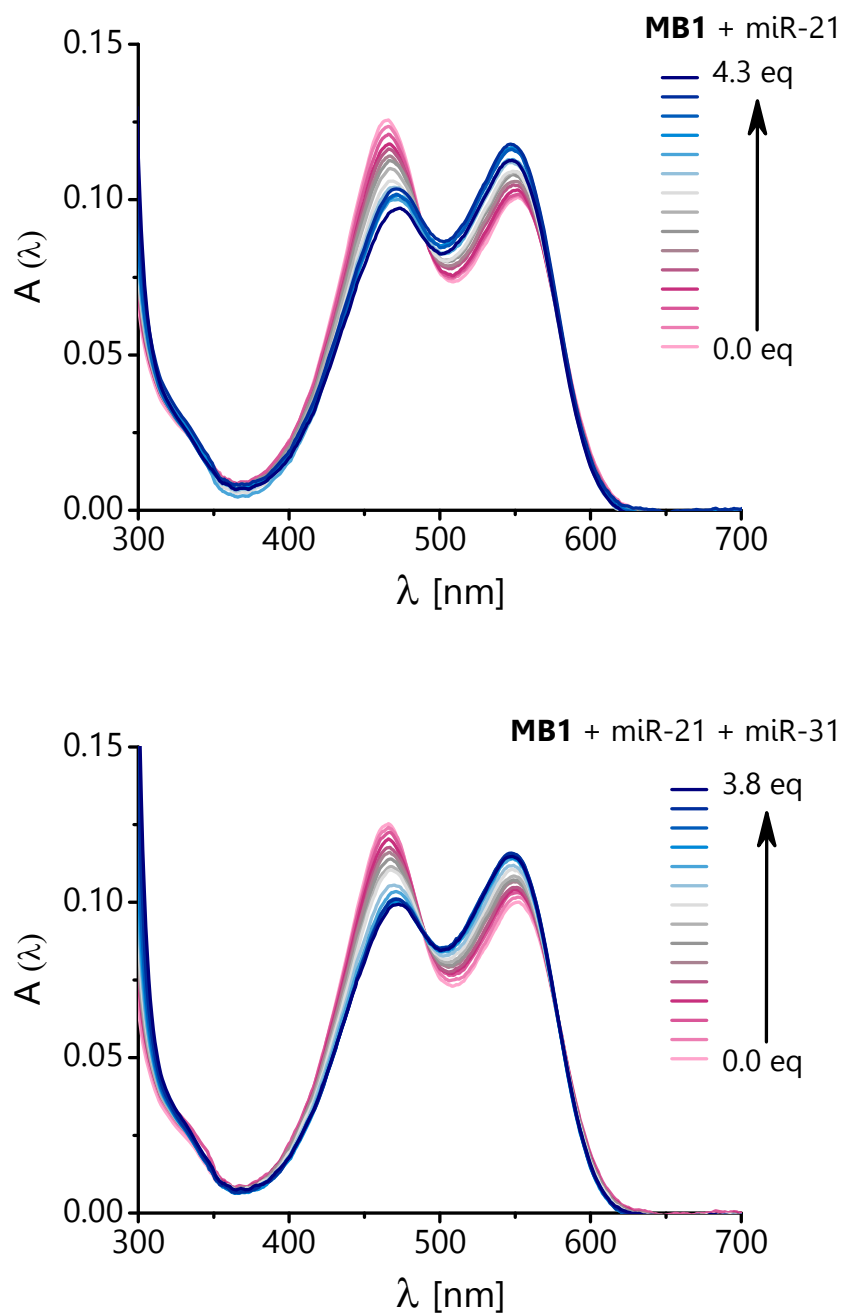


**Figure S5.** Fluorescence spectra after 0 min (start) and after 100 min (end) of endpoint determination measurement of **MB2**.

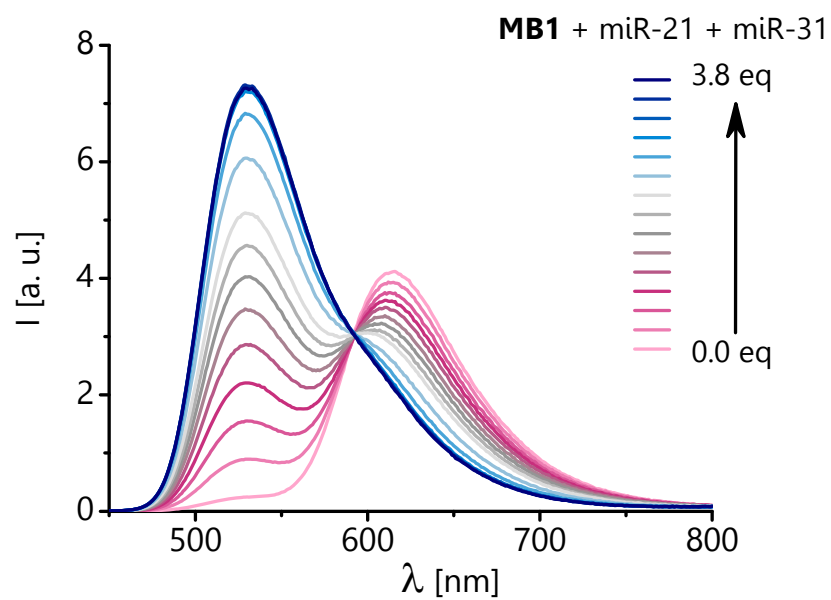
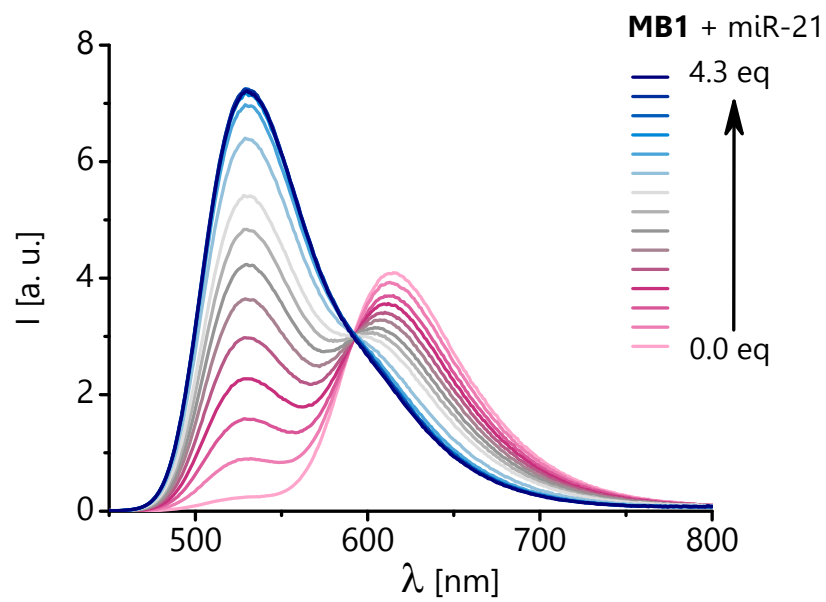


**Figure S6.** Fluorescence intensity changes of dye X<sup>2</sup> (blue) and dye Y<sup>2</sup> (yellow) of endpoint determination measurement of **MB2**.

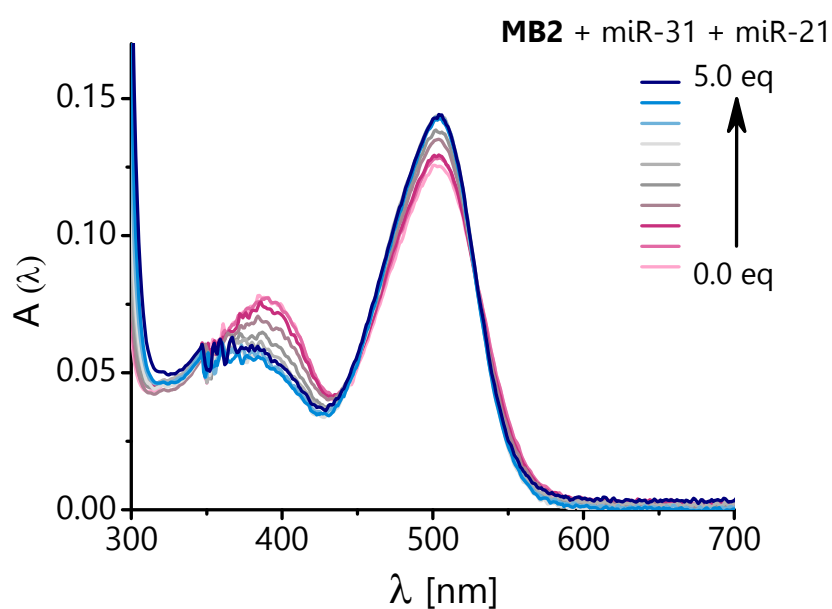
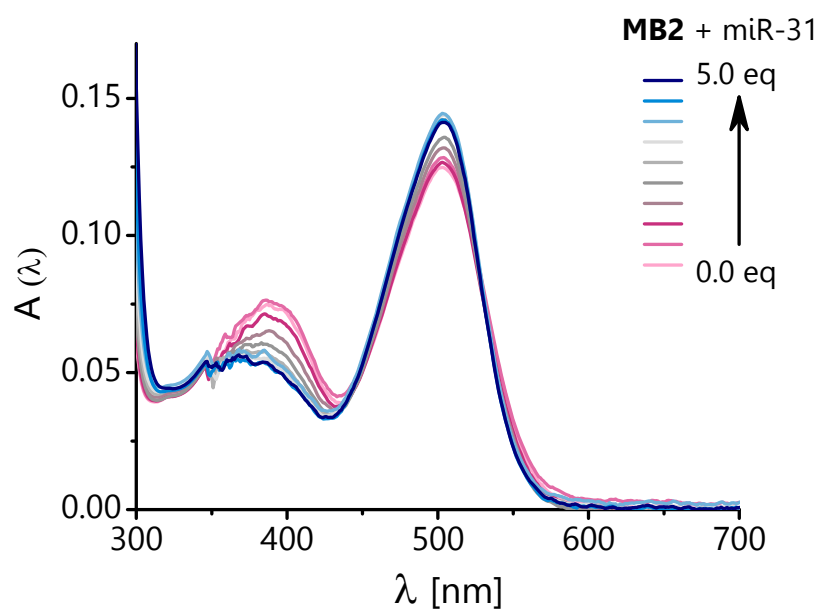
## 7.2 Spectroscopic data of titration experiments



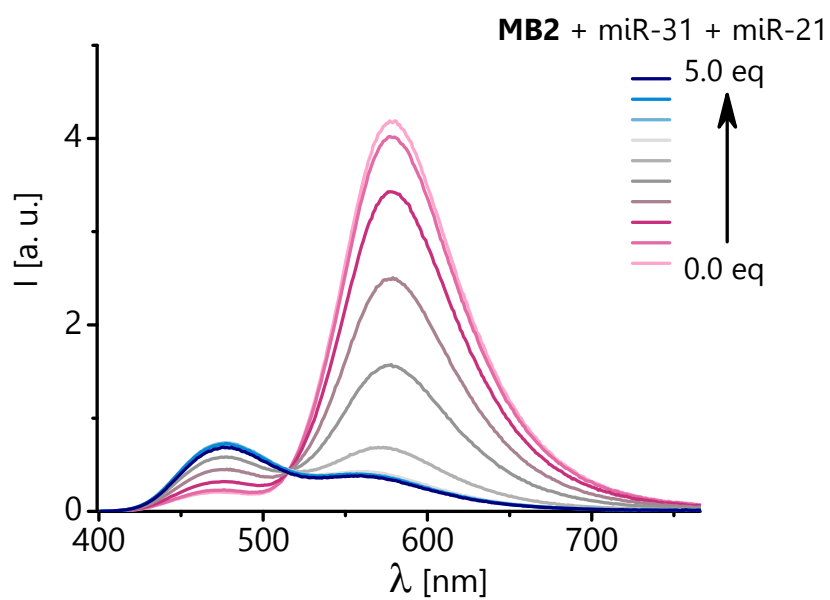
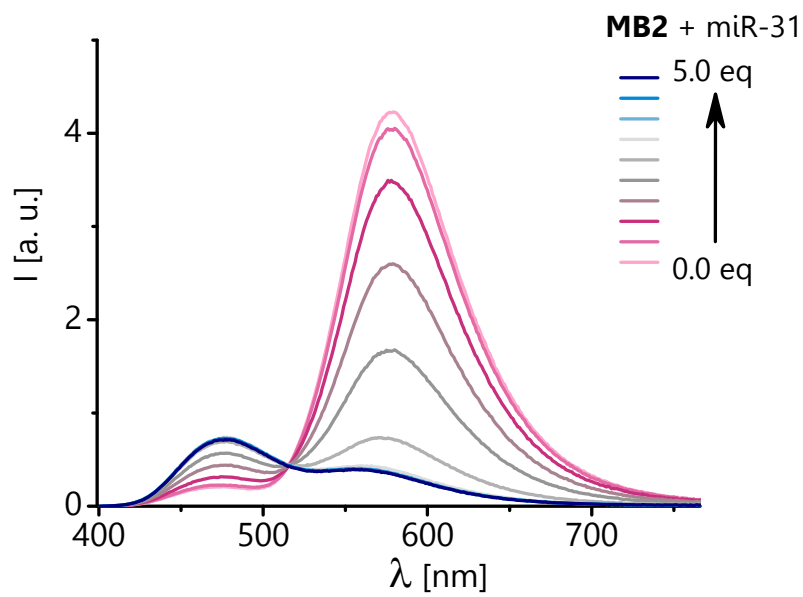
**Figure S7.** Absorption spectra of **MB1** titration experiments with up to 4.3 eq miRNA-target (top) as well as with up to 3.8 eq miRNA-target and additionally up to 3.8 eq “wrong” target miRNA (bottom).



**Figure S8.** Fluorescence spectra of **MB1** titration experiments with up to 4.3 eq miRNA-target (top) as well as with up to 3.8 eq miRNA-target and additionally up to 3.8 eq “wrong” target miRNA (bottom).



**Figure S9.** Absorption spectra of **MB2** titration experiments with up to 5.0 eq miRNA-target (top) as well as with up to 5.0 eq miRNA-target and additionally up to 5.0 eq “wrong” target miRNA (bottom).

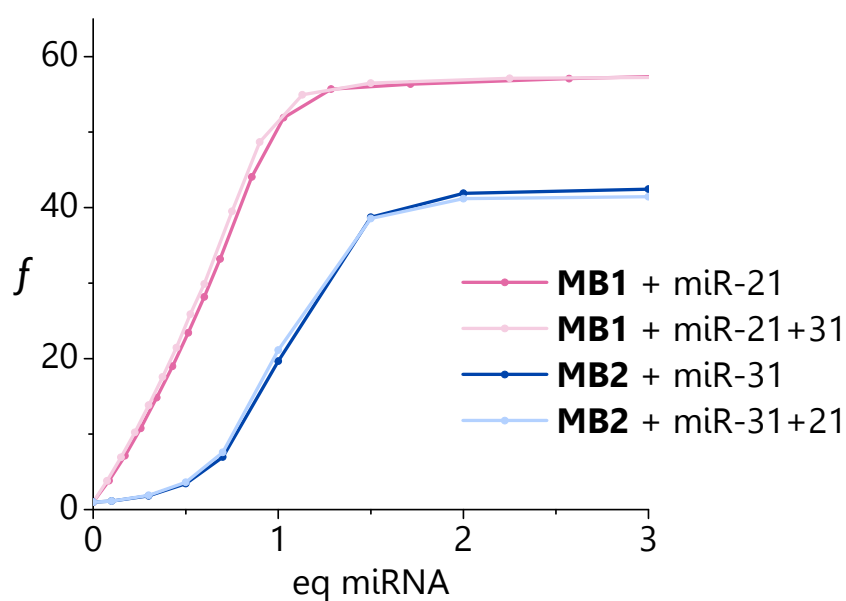


**Figure S10.** Fluorescence spectra of **MB2** titration experiments with up to 5.0 eq miRNA-target (top) as well as with up to 5.0 eq miRNA-target and additionally up to 5.0 eq “wrong” target miRNA (bottom).

### 7.3 Fluorescence enhancement factor

Fluorescence enhancement factors  $f$  were calculated by determined fluorescence intensities of each titration step using following formula.

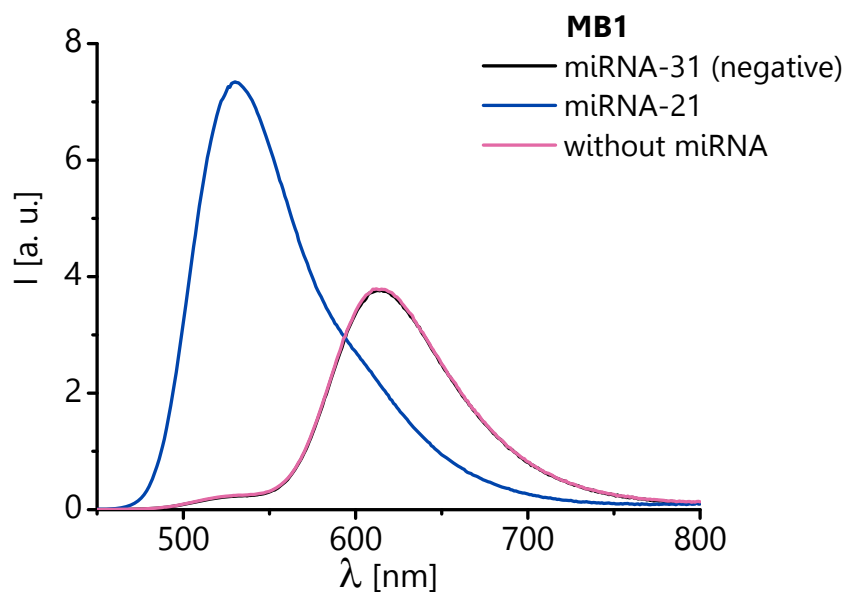
$$f = \frac{I_{Do}/I_{Ak} \text{ (heteroduplex)}}{I_{Do}/I_{Ak} \text{ (hairpin)}}$$



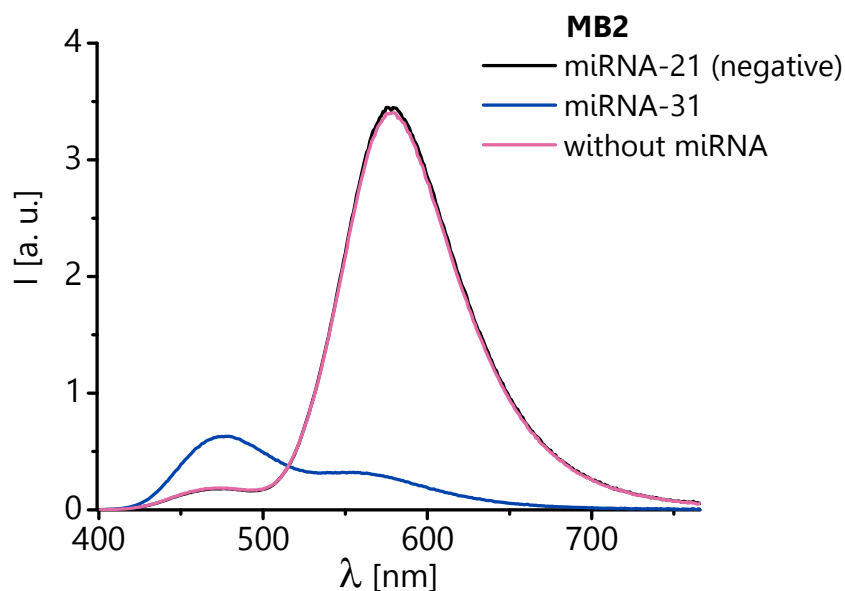
**Figure S11.** Progression of the enhancement factor  $f$  of **MB1** and **MB2** in course of titration with increasing amount of target miRNA as well as increasing amount of both miRNAs.



## 7.4 Spectroscopic data of negative controls



**Figure S12.** Fluorescence spectra of **MB1** without miRNA, with 1.5 equivalents of miRNA-target as well as with 1.5 equivalents “wrong” target miRNA (negative control).



**Figure S13.** Fluorescence spectra of **MB2** without miRNA, with 1.5 equivalents of miRNA-target as well as with 1.5 equivalents “wrong” target miRNA (negative control).

## 8. Cell imaging

### 8.1 Cell culture

The RKO, SW620, WiDr and 239T (mouse embryonic kidney) cell lines were obtained either from the *American Type Culture Collection (ATCC)* or the *Leibniz Institute, German Collection of Microorganisms and Cell Culture (DSMZ), Germany* and cultured routinely in T25 flasks at 37 °C in the presence of 5 % CO<sub>2</sub> and 90 % humidity. Culture media was cell line specific and supplemented with 10 % FCS and 1 % penicillin/streptomycin.

### 8.2 Transfection

Cells were seeded in their respective complete media at a density of  $3 \times 10^5$  and  $1 \times 10^5$  cells/well in 2-well and 4-well *Nunc Lab-Tek* chamber cover-glasses (*Thermo Scientific, Rochester, USA*), respectively. On the day of transfection, the medium was replaced with serum- and phenol-free Gibco Opti-MEM (*Thermo Scientific, Rochester, USA*) after washing the cells with PBS to remove any traces of residual medium. 293T, RKO, WiDr and SW620 cells were transfected with molecular beacon probes for miR-21 (**MB1**) and miR-31 (**MB2**) at an end concentration of 10 nM / MB using the *Metafectane* transfection reagent (*Biontex Laboratories GmbH, Germany*). Double stranded, singly modified DNA (dsDNA) for blue, yellow, green, red and without fluorophores, also transfected at an end concentration of 10 nM were used to optimize the microscopy parameter. The cells were visualized 2 - 6 h following transfection.

### 8.3 RNA extraction, cDNA synthesis and RT-PCR<sup>[5]</sup>

Total RNA including miRNA was extracted and purified from cell lines using the Qiagen's miRNeasy Mini Kit (*Hilden, Germany*) according to the manufacturer's instructions. RNA was reverse transcribed using the miScript II reverse transcription Kit (*Qiagen, Hilden, Germany*). Real-time PCR was performed using Quantitect SYBR Green PCR miScript primers and reagents on an Mx3005P qPCR System (*Agilent Technologies, Santa Clara, USA*). All samples were normalized to two internal controls (RNU6 and SNO72) and fold changes were calculated with the  $2^{-\Delta\Delta C_t}$  method. The Relative expression of miR-21 and miR-31 in the cell lines was used to select cells for the evaluation of these miRNAs with the molecular beacon probes.

**Table S4.** Expression data of cell lines SW620, RKO and WiDr normalized to RNU6, respectively. Standard deviations in parentheses.

miRNA	cell lines			
	Colo-320	SW620	RKO	WiDr
miR-21	1.00 ( $\pm$ 0.11)	49.0 ( $\pm$ 6.70)	44.7 ( $\pm$ 4.05)	139.7 ( $\pm$ 23.1)
miR-31	1.02 ( $\pm$ 0.25)	25.7 ( $\pm$ 1.44)	123.0 ( $\pm$ 7.93)	576.0 ( $\pm$ 93.0)

**Table S5.** Expression data of cell lines SW620, RKO and WiDr normalized to SNO72, respectively. Standard deviations in parentheses.

miRNA	cell lines			
	Colo-320	SW620	RKO	WiDr
miR-21	1.00 ( $\pm$ 0.11)	97.0 ( $\pm$ 13.2)	22.5 ( $\pm$ 2.00)	21.5 ( $\pm$ 3.55)
miR-31	1.02 ( $\pm$ 0.25)	50.7 ( $\pm$ 2.85)	62.1 ( $\pm$ 4.00)	88.6 ( $\pm$ 14.3)

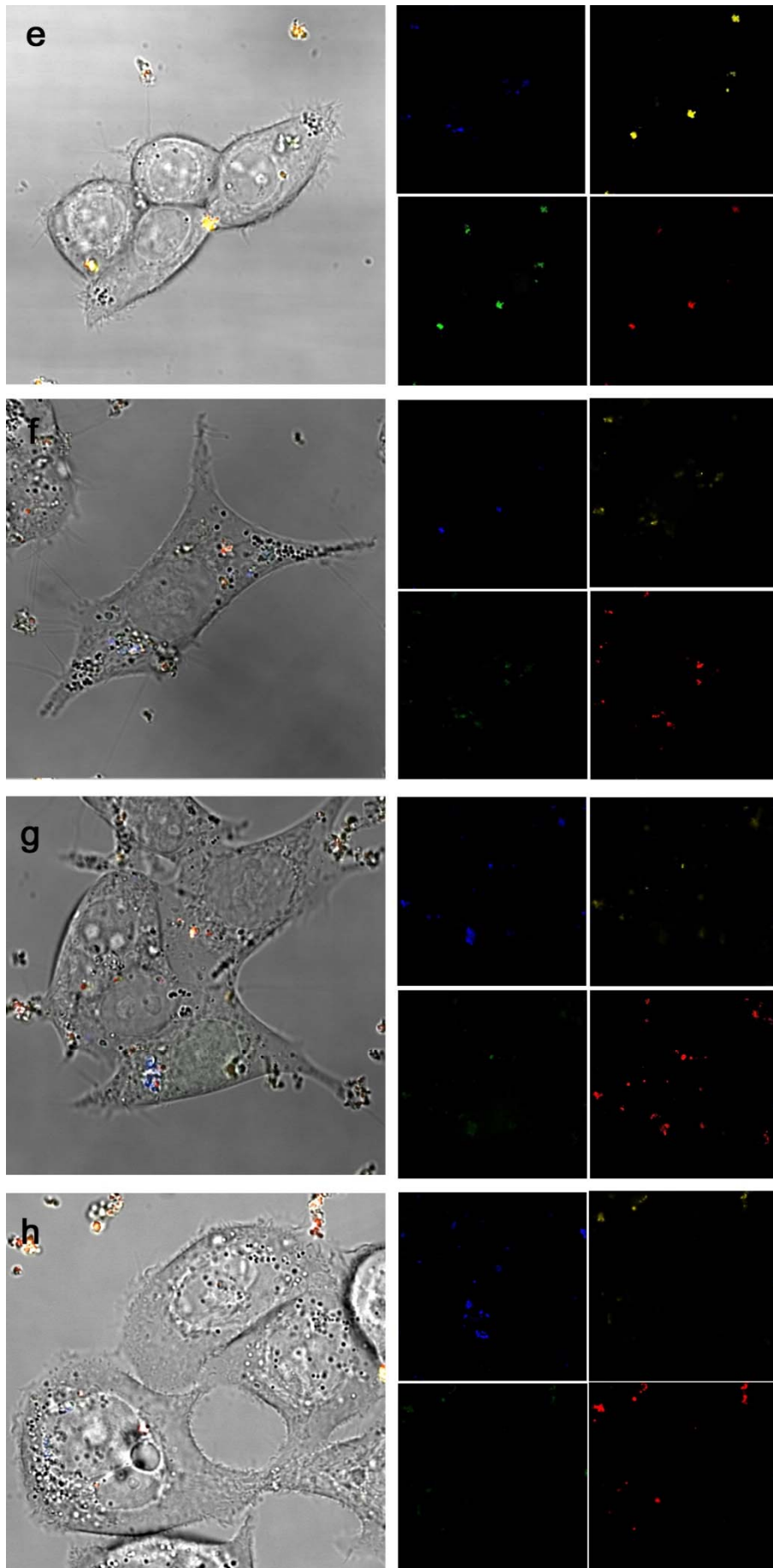
## 8.4 Microscopy

Live cell imaging was performed on a *Leica TCS SP5 II* inverted confocal microscope (*Leica Microsystems GmbH, Wetzlar, Germany*) using a HCX PL APO CS 63.0x1.40 OIL UV objective lens under 'physiological conditions' (37 °C, 5 % CO<sub>2</sub>, humidity). Detection was carried out by sensitive hybrid detectors (HyD) at a lateral pixel resolution of 60.1 nm × 60.1 nm with the detector pinhole being set to 3.0 AU. Additionally, a brightfield image was acquired in each case. The images were post-processed for brightness and contrast with identical settings for the same channels. False color LUTs were applied and the images were superimposed to obtain color images. Due to a time delay of about 11.6 s between the two image pairs, a slight shift of the signals could be observed in some cases.

**MB1** for miRNA-21 was excited at 458 nm, the emission of the donor and the acceptor was recorded instantaneously (line mode) at 520–530 nm and 600–610 nm, respectively. In analogy, **MB2** for miRNA-31 was excited at 405 nm, the emission of the donor and the acceptor was recorded at 520–530 nm and 600–610 nm, respectively.

## 8.5 Cell images

The cell images were evaluated by the *LAS AF Lite* software in the Hyperstack view showing both colorized and composite color modes. All images were recorded and post-processed with identical settings for same channels. Next to the brightfield images of the following figures 1 and 2, respectively are smaller images of the fluorescence detection channels (clockwise: blue, yellow, red, and green).



**Figure S14.** SW620 (e), RKO (f) and (g) and WiDr (h) cells, respectively.

## 8.6 Quantitative Emission Intensity Evaluation

With the aim to evaluate and compare fluorescence intensities of the four emission colors, that have been detected in divers living cancer cell lines, a semi-quantitative intensity-analysis was carried out by using the software LAS AF Lite. All images were post-processed with identical settings for same channels.

In general, to determine the mean value of emission intensities of each channel 1 – 3 cells per image were selected (ROI) and calculated using the quantification software LAS AF Lite. The received raw data were multiplied with color-specific factors (see below) and final values (see Table S6) were plotted in Figure S15 and Figure S16. Thereby calculated cell images in addition to their selected ROIs are shown in Figure S17, respectively.

### Multiplication factors:

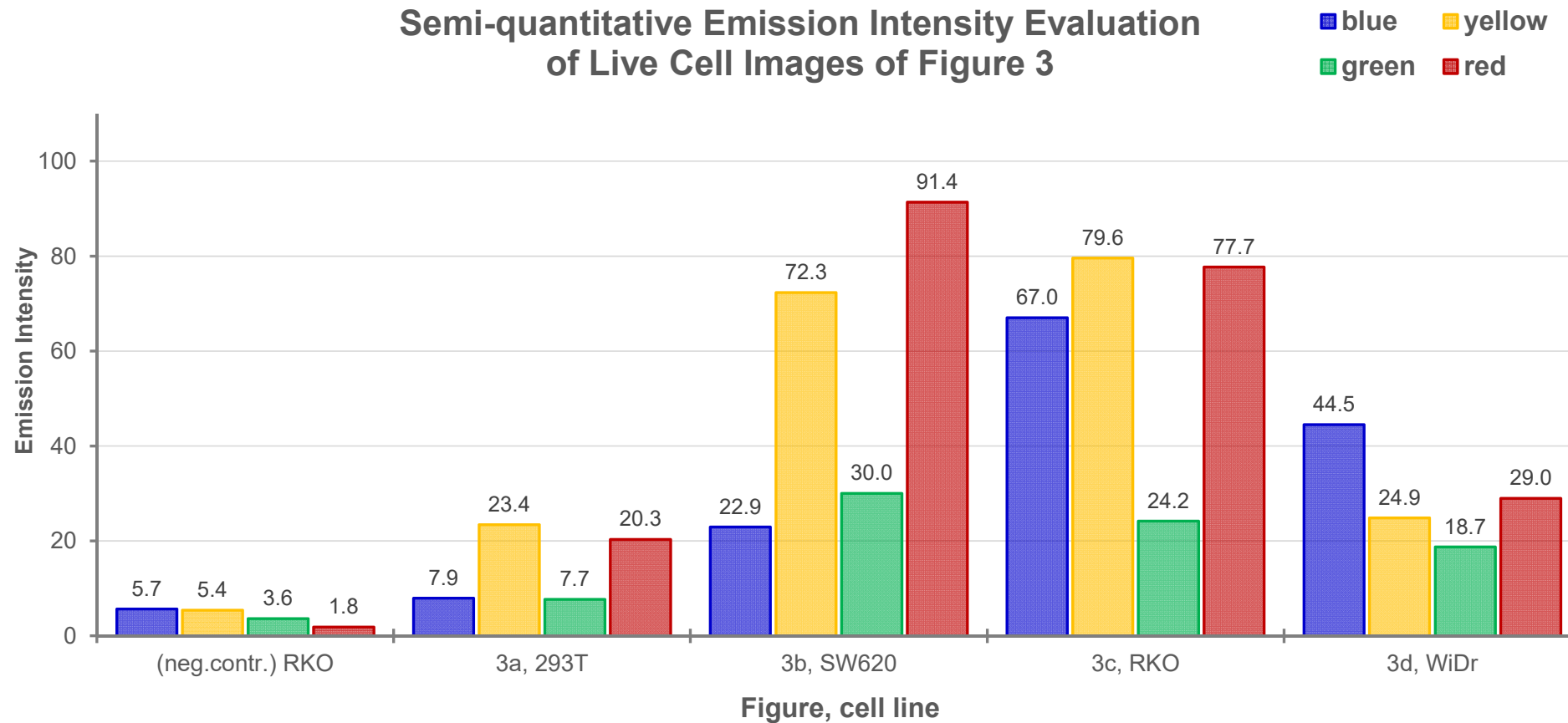
$$f_{\text{blue}} = 0.5 \quad f_{\text{yellow}} = 3 \quad f_{\text{green}} = 1 \quad f_{\text{red}} = 1$$

**Table S6.** Calculated data of the semi-quantitative intensity-analysis of the four emission channels used for imaging of the cell lines 293T, SW620, RKO and WiDr.

	Emission color							
	blue		yellow		green		red	
cell line	Value	multiplied with $f_{\text{blue}}$	Value	multiplied with $f_{\text{yellow}}$	Value	multiplied with $f_{\text{green}}$	Value	multiplied with $f_{\text{red}}$
(neg.contr.) RKO	11.35	5.7	1.8	5.4	3.64	3.6	1.83	1.8
3a, 293T	15.89	7.9	7.8	23.4	7.71	7.7	20.3	20.3
3b, SW620	45.83	22.9	24.1	72.3	30.01	30.0	91.38	91.4
3c, RKO	134.06	67.0	26.52	79.6	24.17	24.2	77.71	77.7
3d, WiDr	89.02	44.5	8.29	24.9	18.7	18.7	28.97	29.0
S14e, SW620	33.98	17.0	33.86	101.6	22.45	22.5	68.82	68.8
S14f, RKO	117.85	58.9	22.65	68.0	32.71	32.7	86.96	87.0
S14g, RKO	93.1	46.6	20.55	61.7	33.97	34.0	74.45	74.5
S14h, WiDr	70.29	35.1	9.69	29.1	15.56	15.6	42.88	42.9

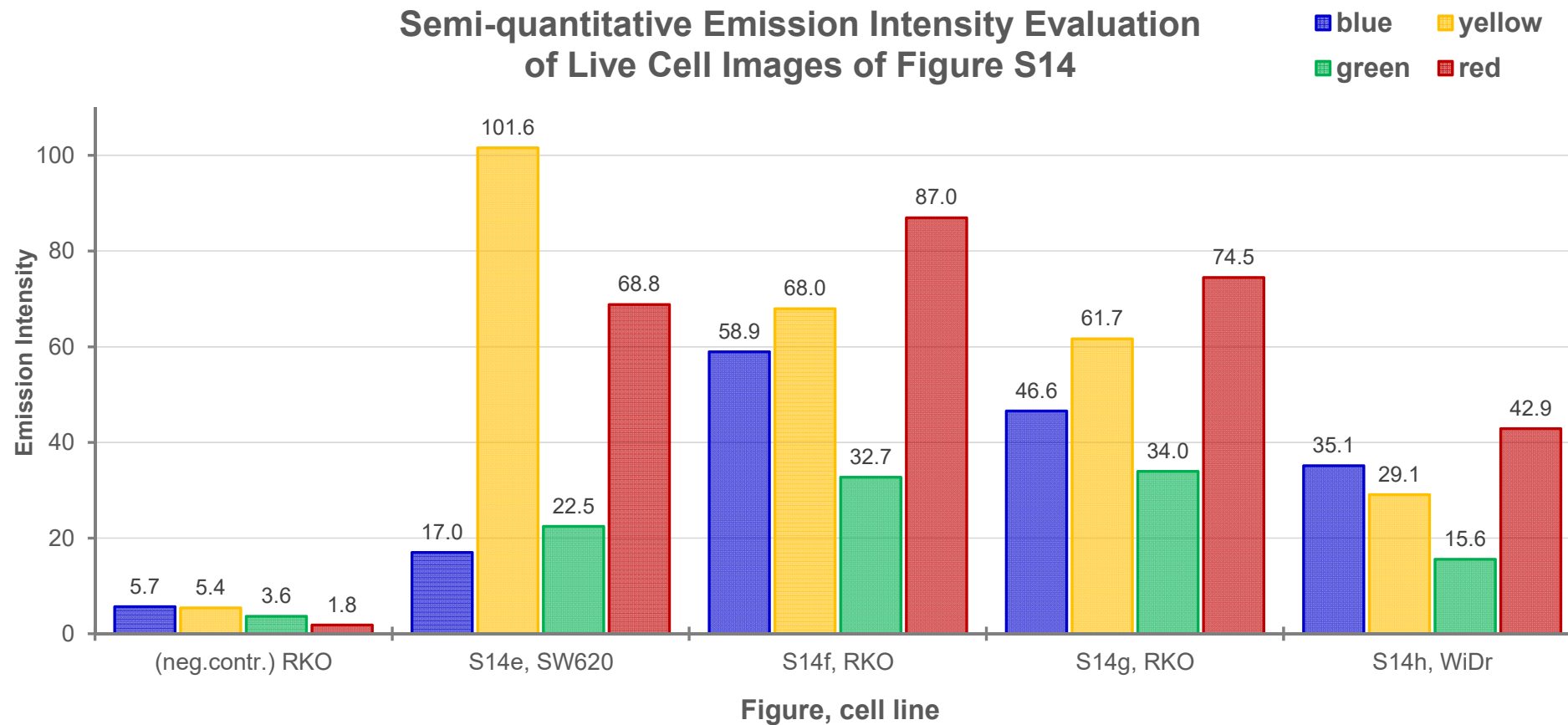


### Semi-quantitative Emission Intensity Evaluation of Live Cell Images of Figure 3

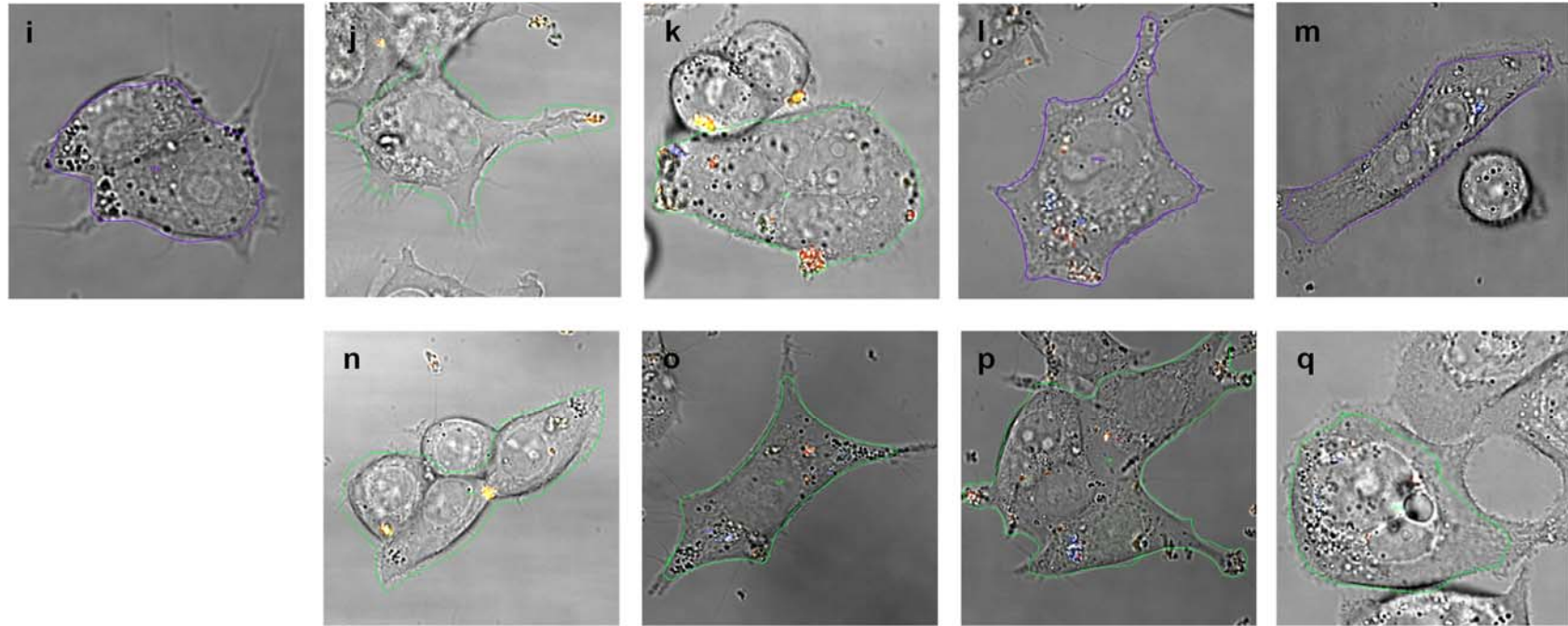


**Figure S15.** Comparison of the semi-quantitative emission Intensity calculation for the cell images of Figure 3 (see manuscript).

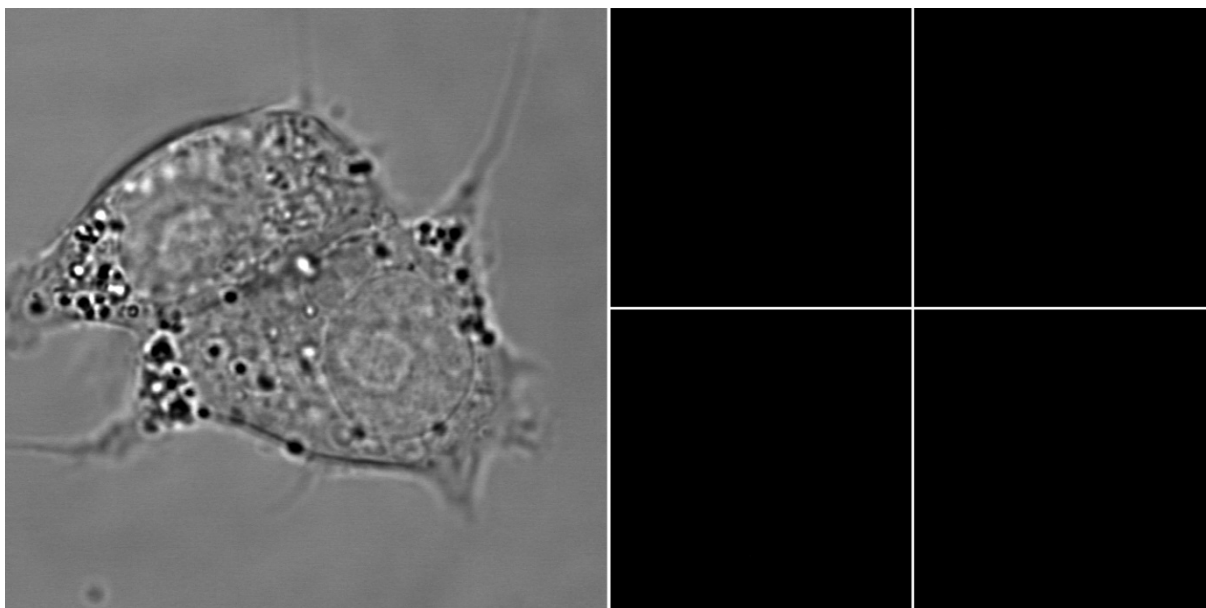
## Semi-quantitative Emission Intensity Evaluation of Live Cell Images of Figure S14



**Figure S16.** Comparison of the semi-quantitative emission Intensity calculation for the cell images of Figure S14 (see Supporting Information).



**Figure S17.** Images of RKO – negative control (i), 293T (j), SW620 (k) and (n), RKO (l), (o) and (p) and WiDr (m) and (q) cells, respectively.



**Figure S18.** Images of RKO cells transfected with unmodified DNA to explore and exclude background fluorescence.

## 9. References

- [1] Bohländer, P. R.; Vilaivan, T.; Wagenknecht, H.-A. *Org. Biomol. Chem.* **2015**, *13*, 9223-9230.
- [2] Bohländer, P. R.; Wagenknecht, H.-A. *Org. Biomol. Chem.* **2013**, *11*, 7458-7462.
- [3] Walter, H.-K.; Bohländer, P. R.; Wagenknecht, H.-A. *ChemistryOpen* **2015**, *4*, 92-96.
- [4] Schulhof, J. C.; Molko, D.; Teoule, R. *Nucleic Acids Research* **1987**, *15*, 397-416.
- [5] Oleksiuk, O.; Abba, M.; Tezcan, K. C.; Schaufler, W.; Bestvater, F.; Patil, N.; Birk, U.; Hafner, M.; Altevogt, P.; Cremer, C. *Oncotarget* **2015**.