Supporting Information

Red-light controlled reagents for micro RNA research

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Materials and methods

Commercially available chemicals of the best quality from Aldrich/Sigma/Fluka (Germany) were obtained and used without purification. Phosphoramidites and controlled pore glass (CPG) solid support were from Aldrich (Germany) and Link Technologies (UK). All media used for cell culture were purchased from Biochrom (Germany). A plate reader Mithras LB940 (Berthold, Germany) was used to measure the chemiluminescence of the Dual-Luciferase Reporter Assay system (Promega GmbH, cat. number E1910). Synthesis of oligonucleotides was conducted on a K&A H-8 DNA /RNA synthesizer. \(^{1}\)H- and \(^{13}\)C-NMR spectra were measured on a Bruker Avance 400 or a Bruker Avance 300 NMR spectrometers. MALDI-TOF mass spectra were recorded on a Shimadzu Axima mass spectrometer. The matrix mixture (2:1 v/v) was prepared from 2',4',6'-trihydroxyacetophenone (THAP, 0.3 M solution in acetonitrile) and diammonium citrate (0.1 M in water). Samples for mass spectrometry were prepared by the dried droplet method using a 1:2 probe/matrix ratio. Mass accuracy with external calibration was 0.1 % of the peak mass, that is ±7.0 at \(m/z\) 7000. HPLC was performed at 22 °C, or at 60 °C with a Varian MethaTerm\textsuperscript{TM} HPLC column temperature controller, on a Shimadzu liquid chromatograph equipped with UV and fluorescence detectors and a Macherey–Nagel Nucleosil C18 250 × 4.6 mm column. Gradient of solution B (CH\(_3\)CN) in solution A [0.1 M aqueous (NEt\(_3\))H(OAc)] was applied to purify conjugates. UV/Vis spectra were measured either on a Cary 100 UV/Vis spectrophotometer (Agilent Technologies, USA) by using quartz glass cuvettes (Hellma GmbH, Germany) with a sample volume of 1 mL or on a Lambda Bio+ UV/Vis spectrophotometer (Perkin Elmer) by using micro cuvettes with a sample volume of 100 µL (BRAND GmbH, Germany). The latter instrument was used for quantification of DNA concentrations, whereas all other experiments were conducted on the former instrument. Fluorescence spectra were acquired on a Varian Cary Eclipse fluorescence spectrophotometer (Agilent Technologies, USA) using fluorescence cuvettes (Hellma GmbH, Germany) with a sample volume of 1 mL. Irradiation experiments were performed with a LED Array 672 (\(\lambda = 650\) nm) from Cetoni GmbH (Germany). Efficiency of small interfering RNAs (siRNAs) in live HeLa cells was quantified by using a Guava easyCyte\textsuperscript{TM} 6-2L Flow cytomter from Merck Millipore. The data were processed with the inCyte\textsuperscript{TM} software package from Merck Millipore.
Syntheses of small molecules

![Synthesis diagram]

Figure S1. Synthesis of 6-N-Benzoxy-5'-O-(9-anthacenyl)-2'-deoxyadenosin-3'-cyanoethyl-N,N-diisopropyl-phosphoramidite.

6-N-Benzoxy-5'-O-(9-anthacenyl)-2'-deoxyadenosin (2)

Anthrone (1.60 g, 8.24 mmol, 2.1 eq) and sodium hydride (60% dispersion in mineral oil, 0.31 g, 7.85 mmol, 2.0 eq) were dissolved in dry dimethyl sulfoxide (40 mL) under nitrogen atmosphere, stirring and at room temperature. Compound 1 (2.01 g, 3.93 mmol, 1.0 eq) was dissolved in dry dimethyl sulfoxide (10 mL) and added dropwise to the red reaction mixture. The reaction mixture was allowed to stir for 24 h under nitrogen atmosphere at 50 °C. Then, CH₂Cl₂ (50 mL) was added and washed with aq. saturated NaCl solution (100 mL). After separating the organic from the aqueous phase, the aqueous phase was extracted three times with CH₂Cl₂ (50 mL). The joined organic phases were washed with water and dried over anhydrous MgSO₄. Next, the solvent was removed under reduced pressure (0.1 mbar) and purification was performed via column chromatography on silica, eluting with a 1/20 (v/v) mixture of methanol/ethyl acetate. After removing the solvent under reduced pressure, 3 was obtained as a yellowish powder (0.37 g, 0.69 mmol, 18%); thin layer chromatography (TLC) on SiO₂, eluent methanol/ethyl acetate (1/20, v/v), Rf = 0.39.
$^1$H NMR (400.05 MHz, Acetone-$d_6$)
$\delta = 9.99$ (bs, 1H), 8.65 (s, 1H), 8.59 (s, 1H), 8.31 (s, 1H), 8.27 (d, $^3$J$_{H-H} = 8.84$ Hz, 2H), 8.10 (d, $^3$J$_{H-H} = 8.59$ Hz, 2H), 7.62-7.59 (m, 1H), 7.54-7.50 (m, 2H), 7.47-7.34 (m, 4H), 6.79 (t, $^3$J$_{H-H} = 6.57$ Hz, 1H), 5.14-5.09 (m, 1H), 4.96 (m, 1H, OH), 4.61-4.58 (m, 1H), 4.53-4.45 (m, 2H), 3.24-3.17 (m, 1H), 2.71-2.65 (m, 1H) ppm.

$^{13}$C {$^1$H} NMR (100.50 MHz, Acetone-$d_6$)
$\delta = 165.97$, 153.17, 152.73, 151.30, 151.22, 143.57, 135.11, 133.40, 133.24, 129.43, 129.32, 129.20, 126.55, 126.30, 126.16, 125.46, 123.37, 123.01, 87.43, 85.49, 76.41, 72.42, 40.47 ppm.

MS (HR-ESI$^+$)
$m/z = 554.1799$ ([M+Na]$^+$) calculated for $C_{31}H_{25}O_4N_5$. $m/z = 554.1796$ ([M+Na]$^+$) found.

Extinction coefficient (Methanol)
$\varepsilon = 7.580$ M$^{-1}$cm$^{-1}$ at $\lambda_{\text{max}} = 368$ nm.

6-N-Benzyoyl-5'-O-(9-anthacenyl)-2'-deoxyadenosin-3'-(2-cyanoethyl)-N,N-diisopropyl-phosphoramidite (3)

Compound 2 (0.18 g, 0.34 mmol, 1.0 eq) and $N,N$-diisopropylethylamine (0.18 g, 0.23 mL, 1.35 mmol, 4.0 eq) were dissolved in dry CH$_2$Cl$_2$ (10 mL) under argon atmosphere, stirring and at room temperature. 2-Cyanoethyl-$N,N$-diisopropylchlorophosphoramidite (0.15 g, 0.68 mmol, 2.0 eq) was added dropwise to the reaction mixture. The reaction mixture was allowed to stir for 2 h under argon atmosphere. Next, the solvent was removed under reduced pressure (0.1 mbar) and purification was performed via column chromatography on silica, eluting with ethyl acetate. After removing the solvent under reduced pressure, 3 was obtained as a yellowish powder (0.20 g, 0.27 mmol, 79 %); thin layer chromatography (TLC) on SiO$_2$, eluent ethyl acetate, $R_f = 0.54$. 

S4
$^1$H NMR (400.13 MHz, Acetone-$d_6$)

$\delta = 9.96$ (bs, 1H), 8.65 (s, 1H), 8.58 (m, 1H), 8.37-8.26 (m, 3H), 8.11 (m, 2H), 8.05 (d, $^3$J$_{HH} = 8.59$ Hz, 2H), 7.64-7.60 (m, 1H), 7.56-7.52 (m, 2H), 7.49-7.37 (m, 4H), 6.84-6.80 (m, 1H), 5.40-5.33 (m, 1H), 4.78-4.71 (m, 1H), 4.62-4.52 (m, 2H), 4.04-3.90 (m, 2H), 3.81-3.75 (m, 2H), 3.44-3.37 (m, 1H), 2.93-2.79 (m, 3H), 1.30-1.26 (m, 12H) ppm.

$^{31}$P NMR (121.49 MHz, Acetone-$d_6$)

$\delta = 150.57, 150.46$ ppm.

MS (HR-ESI$^+$)

$m/z = 754.2877$ ([M+Na]$^+$) calculated for C$_{40}$H$_{42}$O$_5$N$_7$P. $m/z = 754.2887$ ([M+Na]$^+$) found.
Figure S2: $^1$H NMR spectrum of compound 2.

Figure S3: $^{13}$C NMR spectrum of compound 2.
Figure S4: $^1$H NMR spectrum of compound 3.

Figure S5: $^{31}$P NMR spectrum of compound 3.
Singlet oxygen mediated cleavage of compound 2

The decomposition of compound 2 was examined by UV-visible absorbance and fluorescence spectroscopy.

A solution of compound 2 in CH₃OH (1 mM, 1 mL) containing In(III)(pyropheophorbide-a)chloride (InP, 0.1 eq) was irradiated using LED Array 672 (λ = 650 nm, 0.23 W). Before measuring the spectra this solution (100 µL) was diluted with methanol (900 µL). In the UV-visible absorbance spectrum of compound 2, characteristic for the anthracene chromophore peaks were observed in the region between 370 and 410 nm. These spectral features disappeared in the presence of photogenerated ¹O₂ (Figure S2). In the fluorescence spectrum of compound 2 (λₑₓ = 368 nm) characteristic for the anthracene dye peaks were observed in the region between 380 and 440 nm. These spectral features also disappeared in the presence of photogenerated ¹O₂ (Figure 1A, main text of the paper).

Figure S6. Changes of UV-visible absorbance spectra of a mixture of “caged” nucleoside 2 (1 mM) and InP (100 µM) dissolved in CH₃OH upon its irradiation with red light (650 nm, 0.23 W) for the given time periods. The mixture was diluted with CH₃OH 10-fold to acquire the UV-visible absorbance spectra. Compound 2 was found to stable under these conditions in the absence of InP.
Figure S7. Proposed mechanism of uncaging compound 2 in the presence of a photosensitizer InP.

For monitoring cleavage of compound 2 in the presence of $^1$O$_2$ by $^1$H-NMR spectroscopy a solution of compound 2 (15 mM) with InP (0.1 eq) was prepared in CDCl$_3$ containing DMSO-d$_6$ (1 %, v/v) and acetic acid (1 %, v/v). To generate $^1$O$_2$ the resulting mixture was exposed to red light ($\lambda = 650$ nm, 0.29 W) for the given time periods.

In the presence of 1% acetic acid (v/v) endoperoxide 6 is formed, which then decomposes to 6-N-Benzoyl-2'-deoxyadenosin (7) and anthraquinone (8) (Figures S7, S8).
Figure S8: $^1$H NMR spectra (lower three spectra) of a mixture of 2 (15 mM) and InP (0.1 eq) in CDCl$_3$ containing additionally 1% DMSO-$d_6$ (v/v) and 1% acetic acid, which was irradiated with red light (650 nm, 0.29 W) for the time periods indicated on the plot. Other experimental conditions are described in the text above this plot.
Synthesis of chemically modified RNAs

Oligoribonucleotides were synthesized on an H-8 DNA/RNA synthesizer on a 1 µmol-scale by using the standard (3′→5′) synthesis and in accordance to the recommendations of the manufacturers. Commercially available 2′-O-TC RNA phosphoramidites (rA-Bz, rC-Ac, rG-ib, rU; Aldrich, Germany) as well as DNA phosphoramidites (dT-CE; Link Technologies, Scotland) and solid supports (3′-Amino-Modifier C7 CPG; Link Technologies, Scotland) were used in the 3′→5′ synthesis. Oligoribonucleotides were cleaved from the solid support and deprotected by the treatment with ethylenediamine at room temperature for 2 h.

After removal of ethylenediamine with anhydrous toluene, the RNA samples were washed out from the support with a (NH₄)OAc aqueous solution (150 mM) buffered at pH 7.4 and purified by HPLC by using the following gradients of solution B (CH₃CN) in solution A (0.1 M aqueous (NEt₃H)(OAc)):

**Gradient A** for 2 min 0% solution B, in 40 min from 0 to 60% solution B, in 1 min from 60 to 90% solution B, for 7 min 90% solution B, in 1 min from 90% to 0% solution B.

**Gradient B** for 2 min 0% solution B, in 40 min from 0 to 60% solution B, in 10 min from 60 to 90% solution B, for 10 min 90% solution B, in 3 min from 90% to 0% solution B.

The new conjugates were identified by MALDI-TOF mass spectrometry and their purity was confirmed by analytical HPLC. Purified RNAs were dissolved in ammonium acetate buffer (150 mM (NH₄)OAc in RNase-free water, pH 7.4) and stored at -25 °C.

**Synthesis of 3′-P1- or P2-modified lagging RNAs:** 3′-Amino-Modifier C7 CPG solid support containing a DMT protecting group and an amino group protected with fluorenlymethoxycarbonyl group (Fmoc) (30 mg, 1 µmol of the Fmoc-group on the solid support) was treated four times with DBU in DMF (2 % v/v, 1 mL) and was shaken each time for 60 min. Then the solid support was washed with anhydrous DMF (3×1 mL), anhydrous CH₃CN (2×1 mL) and lyophilized overnight. The photosensitizers (pyropheophorbide-a (P1) or In³⁺(pyropheophorbide-a)chloride (P2), 1.0 eq, 16.5 µmol), N,N,N’,N’-tetramethyl-O-(1H-benzotriazol-1-yl)uronium
hexafluorophosphate (HBTU; 1.8 eq, 11.4 mg, 30 μmol), and 1-hydroxy-1H-benzotriazole (HOBT; 2.0 eq, 4.5 mg, 33 μmol) were dissolved in anhydrous DMF (0.6 mL) and N,N-diisopropylethylamine (DIPEA; 4.5 eq, 12.9 μL, 74.3 μmol) was added. This solution was immediately added to the solid support and shaken for 16 hours. The obtained green slurry was washed with DMF (3×1 mL), CH₃CN (2×1 mL) and lyophilized overnight. The solid support was then used for the standard (3’→5’) RNA synthesis.

Figure S9: Synthesis of 3’-P1 or P2-modified lagging RNAs.

**Synthesis of 5’ anthracenyl “caged” guide RNAs:** Synthesis of the RNA until the penultimate nucleobase was conducted on the DNA/RNA synthesizer, including the 5’-end DMT deprotection. 5’-O-(9-anthracenyl)-2’-deoxynucleoside-3’-O-phosphoramidite (either compound 3 (Scheme 1) or its thymidine analogue reported previously,¹⁵ 45.0 μmol) was dried for one day in vacuum (0.01 mbar), dissolved in anhydrous acetonitrile (0.3 mL) and separated evenly in three portions. Immediately before coupling 5-(ethylthio)tetrazole solution (0.25 M, 0.1 mL, Link Technologies) was added to the phosphoramidite solution and the mixture obtained was added to the RNA-containing solid support. After 5 min coupling the solution was removed from the solid support, washed with anhydrous CH₃CN (2×0.2 mL). Then, the next portion was added, reacted and worked up as described above. This process was repeated one more time with the third portion of the phosphoramidite. Then, the solid support was oxidized with 0.02 M iodine in THF (0.3 mL, Proligo), washed with CH₃CN (2×0.2 mL) and lyophilized overnight. Deprotection and cleavage from the
solid support, as well as purification was performed as described above for modified lagging RNAs.

**EGFR-siRNA, guide strand (RNA1):** HPLC gradient A, $R_t = 13.13$ min; MALDI-TOF MS, negative mode, calculated for $C_{197}H_{248}N_{66}O_{147}P_{20}$ ([M-H]): 6510.94 m/z, found 6514.33 m/z; yield = 14.02 %.

**EGFR-siRNA, “caged” guide strand (RNA1*):** HPLC gradient A, $R_t = 19.41$ min; MALDI-TOF MS, negative mode, calculated for $C_{211}H_{256}N_{66}O_{147}P_{20}$ ([M-H]): 6690.97 m/z, found 6689.59 m/z; yield = 2.76 %.

**miR20a, guide strand (RNA2):** HPLC gradient B, $R_t = 14.46$ min; MALDI-TOF MS, negative mode, calculated for $C_{222}H_{272}N_{90}O_{158}P_{22}$ ([M-H]): 7409.51 m/z, found 7410.05 m/z; yield = 1.93 %.

**miR20a, “caged” guide strand (RNA2*):** HPLC gradient B, $R_t = 21.93$ min; MALDI-TOF MS, negative mode, calculated for $C_{236}H_{280}N_{90}O_{158}P_{22}$ ([M-H]): 7585.73 m/z, found 7581.06 m/z; yield = 12.05 %.

**EGFR-siRNA, lagging strand (RNA3):** HPLC gradient A, $R_t = 13.17$ min; MALDI-TOF MS, negative mode, calculated for $C_{204}H_{251}N_{83}O_{142}P_{20}$ ([M-H]): 6756.16 m/z, found 6758.98 m/z; yield = 11.68 %.

**EGFR-siRNA, P2-modified lagging strand (RNA4):** HPLC gradient A, $R_t = 32.36$ min; MALDI-TOF MS, negative mode, calculated for $C_{244}H_{297}N_{88}O_{148}P_{21}InCl$ ([M-Cl]): 7594.80 m/z, found 7596.90 m/z; yield = 1.56 %.

**miR20a, lagging strand (RNA5):** HPLC gradient B, $R_t = 13.11$ min; MALDI-TOF MS, negative mode, calculated for $C_{216}H_{270}N_{80}O_{159}P_{22}$ ([M-H]): 7211.36 m/z, found 7210.38 m/z; yield = 1.55 %.

**miR20a, P1-modified lagging strand (RNA6):** HPLC gradient B, $R_t = 39.66$ min; MALDI-TOF MS, negative mode, calculated for $C_{256}H_{318}N_{85}O_{165}P_{23}$ ([M-H]): 7937.19 m/z, found 7938.86 m/z; yield = 0.93 %.
miR20a, **P2-modified lagging strand (RNA7)**: HPLC gradient B, $R_t = 35.15$ min; MALDI-TOF MS, negative mode, calculated for $C_{256}H_{316}N_{85}O_{165}P_{23}InCl$: $8050.99 \, m/z$, found $8051.02 \, m/z$; yield = 1.45%.

![HPLC profiles of analytically pure RNA conjugates.](image)

**Figure S10.** HPLC profiles of analytically pure RNA conjugates.

### Annealing of RNAs

Double-stranded siRNAs/miRNAs of desired concentrations were prepared in RNase-free water with 20 volume percent of 5X siRNA annealing buffer (100 mM KCl, 2 mM MgCl$_2$, 25 mM KOH, 30 mM HEPES in RNase-free water, pH 7.4; all bio grade, Sigma). The mixtures were heated up to 90 °C and then slowly (5 °C/min) cooled to 22° C. The duplexes annealed in such a way were used in all further experiments.

### Irradiation of RNAs with red light

The irradiation of dsRNAs was examined by analytical HPLC (gradient A). A solution of the desired oligonucleotide (50 µM) was prepared in the annealing buffer (12 µL)
and annealed as previously described. To generate $^{1}\text{O}_2$ the resulting mixture was exposed to red light ($\lambda = 650\text{ nm}, 0.23\text{ W}$) for given time periods. Then the mixtures obtained (10 $\mu$L) were analyzed by HPLC at 60 °C. The HPLC data confirm, that RNA1*/RNA4 is fully uncaged by red light to yield the active siRNA RNA1/RNA4 (Figure 1C, main text of the paper). Similarly, uncaging of RNA2*/RNA6 and RNA2*/RNA7 occurs with formation of desired active compound RNA2. However, in the latter case substantial amount of non-identified product (indicated with * in HPLC profiles shown in Figure S11) was formed. According to UV-visible spectroscopy, this product does not contain anthracene chromophore, but contains RNA-nucleobases.

![HPLC profiles](image)

Figure S11. HPLC profiles of “caged” miR-20as RNA2*/RNA6 (30 $\mu$M) and RNA2*/RNA7 (50 $\mu$M) dissolved in HEPES buffer (6 mM) containing KCl (20 mM), MgCl$_2$ (0.4 mM) adjusted to pH 7.4 with KOH (5 mM)), which were either kept in the dark or irradiated for 30 min with an LED light source (650 nm, 0.23 W). HPLC was conducted at denaturing conditions (60 °C): peak “*” corresponds to an unidentified side product.

**Cellular assays**

**Cells and cell culture**

The human cervical cancer cells line (HeLa TK) was cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10 % fetal bovine serum (FBS), 1 % L-glutamine, and 1 % penicillin/streptomycin. 24 h before transfection the cells were detached from the surface by removing the medium, washing the cells with DPBS
(2x10 ml) and addition of 1.5 ml trypsin (0.05 %)/ethylenediamine tetracetic acid (EDTA, 0.02 %) solution directly to the cells. The cells were incubated for 2 min at 22°C, the trypsin/EDTA solution was removed and the dry cells were incubated for 5 min at 37 °C, 95 % air humidity and 5 % CO₂. Then, the cells were re-suspended in the fresh medium and diluted with the medium to 80 cells/µL. Next, 0.5 mL aliquots of such suspensions were placed into wells of 24-well plates and the cells were allowed to get attached to the surface for 24 h in the incubator at 37 °C, 95 % air humidity and 5% CO₂ content.

Plasmids

Dual-luciferase reporter plasmid (psiCheck2) with miR20a binding site was used to measure the intracellular activity of the synthesized and annealed microRNA. Briefly luciferase was used as reference and renilla luciferase as target. Lipofectamine™ 2000 (Invitrogen) and Lipofectamine™ 3000 (Invitrogen) was used as transfection reagent for plasmids, microRNA and siRNA.

Inhibition of EGFR expression by red-light activated siRNA

First, a mixture of siRNA (3 pmol, 1.5 µL) was prepared in the reduced serum medium (Opti-MEM, 23.5 µL). Then, Lipofectamine™ 3000 (1.0 µL) was mixed with Opti-MEM (24.0 µL) and incubated for 5 min at 22°C. The resulting solution was added to the siRNA mixture and incubated for another 10 min at 22°C. For this cell experiments 24-well plates were used. The medium was removed, washed with Dulbecco’s phosphate-buffered saline (DPBS, 2x500 µL) and replaced, for each well, with Dulbecco’s modified eagle medium (DMEM) without phenol red (400 µL), containing 1 % L-glutamine and 10 % FBS, and the transfection mixtures (50 µL). The cells were incubated for 24 h at 37 °C, 95 % air humidity and 5 % CO₂. If necessary, the cells were irradiated with red light (λ = 650 nm, 0.23 W) after 2 h of incubation. 3 h prior to internalization studies the cells were starved. The medium was removed, the cells washed with DPBS (2x500 µL) and starvation medium (DMEM supplemented with 1 % L-glutamine and 1 % penicillin/streptomycin) was added. Epidermal Growth Factor biotinylated, complexed to Alexa Fluor® 488 Streptavidin (150 ng, Alexa Fluor® 488 EGF complex, Life Technologies, cat.
Figure S12: Flow cytometric histograms of cells transfected with 150 ng Alexa Fluor® 488 EGF complex (green-colored area); A) RNA1/RNA3 (3 pmol, 0 min, red-colored area) and RNA1/RNA3 (3 pmol, 5 min, blue-colored area); B) RNA1*/RNA4 (3 pmol, 0 min, red-colored area) and RNA1*/RNA4 (3 pmol, 5 min, blue-colored area); C) RNA1*/RNA3 (3 pmol, 0 min, red-colored area) and RNA1*/RNA3 (3 pmol, 5 min, blue-colored area); D) RNA1/RNA4 (3 pmol, 0 min, red-colored area) and RNA1/RNA4 (3 pmol, 5 min, blue-colored area). Data obtained for untreated cells are indicated with the black trace (transparent area). In these plots the intensity of the fluorescence of cells (Green Fluorescence) monitored using excitation at $\lambda_{\text{ex}} = 488$ nm and emission at $\lambda_{\text{em}} = 525 \pm 30$ nm is plotted on the X-axis and cell count on Y-axis.

Number E-13345) was added directly to the starvation medium and incubated for 15 minutes at 37°C. The medium was removed, washed with DPBS (2x500 µL) and membrane bound EGF-Alexa488 was removed by washing cells with acid-stripping buffer (50mM Glycine, 150mM NaCl, pH 3.0) for 1 min at room temperature. For the
measurement of the siRNA activity via flow cytometry the cells were detached from the surface by removing the acid-stripping buffer, washing the cells with DPBS (2x500 µL) and addition of 100 µL trypsin (0.05 %)/ethylenediamine tetracetic acid (EDTA, 0.02 %) solution to each well. The cells were incubated for 2 min at 22°C, the trypsin/EDTA solution was removed and the dry cells were incubated for 5 min at 37 °C, 95 % air humidity and 5 % CO₂. Then, the cells were re-suspended in 400 µL of albumin bovine serum (2 %)/sodium azide (0.2 %) in DPBS, transferred to 1.5 mL Eppendorf tubes and centrifuged for 3 min at 22 °C (1000 rcf). The supernatant was removed and 100 µL of paraformaldehyde (0.08 %) in DPBS was added to each tube for fixation of the cells. The probes were gently vortexed and the fluorescence of the cells was immediately measured via flow cytometry. For fluorescence detection of Alexa Fluor® 488 the blue laser (λ<sub>ex</sub> = 488 nm) was used for excitation and the green filter (525/30 nm) was used (Figure S12).

Inhibition of renilla luciferase expression by red-light activated microRNA 20a

First, a mixture of microRNA (1 pmol, 5.0 µL) and psiCheck2 plasmid (20 ng, 2.0 µL) was prepared in the reduced serum medium (Opti-MEM, 43 µL). Then, Lipofectamine™ 2000 (1.0 µL) was mixed with Opti-MEM (49.0 µL) and incubated for 5 min at 22°C. The resulting solution was added to the siRNA/plasmid mixture and incubated for another 20 min at 22°C. For this cell experiments 24-well plates were used. The medium of the cells was removed, washed with DPBS (2x500 µL) and replaced, for each well, with DMEM without phenol red (400 µL), containing 1 % L-glutamine, and the transfection mixtures (100 µL). The cells were incubated for 24 h at 37 °C, 95 % air humidity and 5 % CO₂. If necessary, the cells were irradiated with red light (λ = 650 nm, 0.23 W) after 2 h of incubation. For the measurement of the microRNA activity by chemiluminescence of the dual-luciferase reporter assay system, the cells were detached from the surface by removing the medium, washing the cells with DPBS (2x500 µL) and addition of passive lysis buffer (100 µL, 20 volume percent of lysis buffer X5 in DPBS). The well plates were gently shaken for 15 minutes. The lysate of each well (50 µL) was transferred to a 96-well plate and diluted with passive lysis buffer (50 µL). The chemiluminescence was measured with the help of a Mithras LB940 plate reader (Berthold) and a Dual-Luciferase Reporter-Kit (Promega). The addition of the desired reagents was performed by the plate reader. First, luciferase assay reagent II (25 µL/Well) was added, the well plate
shaken for 3 seconds and then firefly luciferase activity (reference) quantified by chemiluminescence measurement for 12 seconds. Next, Stop&Glo reagent (25 ul/Well) was added, the well plate shaken for 3 seconds and then renilla luciferase activity (target) quantified by chemiluminescence measurement for 12 seconds.