Electronic Supplementary Information

$g\text{-}C_3N_4$ nanosheets-based ratiometric fluorescent probe for the amplification and imaging of miRNA in living cells

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Electronic Supplementary Information Included:

Figure S4. The variation of fluorescence intensity of BCNNS (100 μg mL^{-1}) as a function of ionic strength with excitation at λ_{ex} 340 nm.

Figure S5. The variation of fluorescence intensity of BCNNS (100 μg mL^{-1}) as a function of pH value with excitation at λ_{ex} 340 nm.

Figure S6. The variation of fluorescence intensity of BCNNS (100 μg mL^{-1}) as a function of irradiation time with excitation at λ_{ex} 340 nm.

Figure S7. Quenching effect of BCNNS at various concentrations on CuNCs. The concentration of CuNCs is 100 nmol L^{-1}. BCNNS concentrations are given within a range of 0-200 μg mL^{-1}. The excitation and emission wavelengths are set at 340 and 600 nm, respectively.

Figure S8. Quenching effect of BCNNS on the fluorescence of CuNCs with various concentrations. The concentration of BCNNS is 200 μg mL^{-1}. CuNCs concentrations are given in a range of 0-200 nmol L^{-1}. The excitation and emission wavelengths are set at 340 and 600 nm, respectively.

Figure S9. Time-dependent fluorescence changes of BCNNS-CuNCs in the presence of miR-582-3p (10 pmol L^{-1}). The excitation and emission wavelengths are set at 340 and 600 nm, respectively.

Figure S10. The fluorescence response of the system in the presence of miR-582-3p (10 pmol L^{-1}) under different conditions: BCNNS-H1/H2-CuNCs; BCNNS-H1-CuNCs; BCNNS-H2-CuNCs; BCNNS-H1/H2-CuNCs-miR-582-3p; BCNNS-H1-CuNCs-miR-582-3p; H1/H2-CuNCs-miR-582-3p and BCNNS-miR-582-3p. The concentrations of H1/H2-CuNCs, H1-CuNCs and H2-CuNCs are 100 nmol L^{-1}; the concentration of BCNNS is 200 μg mL^{-1}.

Figure S11. Effect of the BCNNS-CuNCs concentration (0, 2, 5, 10, 20, 50, 100, 200 and 500 μg mL^{-1}) on the viability of A549 cells with an incubation

time of 24 h.

Figure S12. Effect of the BCNNS-CuNCs concentration (0, 2, 5, 10, 20, 50, 100, 200 and 500 μg mL^{-1}) on the viability of BEAS-2B cells with an incubation time of 24 h.

Figure S13. Confocal images of A549 cells incubated with BCNNS-CuNCs (200 μg mL^{-1}) for 0, 1, 2, 3, 4 and 5 h. Scale bar: 40 μm.

Figure S14. The calibration curve between miR-582-3p mimics concentration and relative optical density (red channel *vs* blue channel).

1. Experimental details

1.1 Chemicals and materials.

All the nucleic acids used in this study were custom-designed and synthesized by Sangon Biological Engineering Technology & Services Co. Ltd (Shanghai, China). The sequences of nucleic acids were listed in Table S1. Thiourea, H_3BO_3, $CuSO_4 \cdot 5H_2O$, NaCl, KCl, $MgCl_2$, NaOH, HCl, H_3PO_4, CH_3COOH, $NaHPO_4 \cdot 12H_2O$, KH_2PO_4, HNO_3, dimethyl sulfoxide (DMSO) were provided by Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). 3-Morpholinopropanesulfoinc acid (MOPS) and tris-base were the products of Aladdin Bio-Chem Technology Co., Ltd (Shanghai, China). Dulbecco's modified Eagle's medium (DMEM, high glucose), fetal bovine serum, penicillin, streptomycin and trypsin (0.25%) were obtained from Hyclone (Thermo Scientific, USA). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay kit was acquired from KeyGEN Biotech Co. Ltd (Nanjing, China). Entranster^{TM}-R4000 transfection reagent was purchased from Engreen Biosystem Co, Ltd (Beijing, China). All the chemicals were used as received and deionized water (18 MΩ cm^{-1}) was used throughout.

1.2 Instrumentations.

The morphology of copper nanocluster (CuNCs) and boron doped g-C_3N_4 nanosheets (BCNNS) were studied by a JEM-ARM 200F high-resolution transmission electron microscope (JEOL Ltd.) and a Tecnai G220 field-emission electron microscope (FEI, America). Atomic force microscopy (AFM) image was acquired on a Bruker Dimension icon atomic force microscopy (Bruker, Germany)

using tapping mode. X-ray diffraction (XRD) was characterized with an MPDDY2094 X-ray diffractometer (PAnalytical B.V., Netherlands) with Cu-Kα irradiation (λ=1.5406 Å) in a range of 2θ=5-80°. X-ray photoelectron spectra (XPS) analyses were carried out on an ESCALAB 250 X-ray photoelectron spectroscopy (Thermo Instruments Inc., USA) with monochromated Al Kα 280.00 eV excitation source. UV-vis absorption spectra were measured on a U-3900 spectrophotometer (Hitachi High Technologies, Japan). Fluorescence (FL) measurements were carried on an F-7000 fluorescence spectrometer (Hitachi High Technologies, Japan) with a 0.5 cm optical path, a scan speed of 1200 nm min^{-1} and 10.0 nm slits for both excitation and emission. Inductively coupled plasma mass spectrometer (ICP-MS, Agilent 8900, Agilent Technologies, CA, USA) was used for the quantification of target miRNA by detecting the isotope of ^{63}Cu. MTT assay was performed on a Synergy H1 ELISA plate reader (BioTek Instruments Inc., USA). Fluorescence images of cells were taken by an FV 1200 confocal laser scanning microscopy (Olympus, Japan) with a 40×objective lens.

2. Supplemental tables and figures

Table S1. Sequences of nucleic acid.

Name	Sequence (5'-3')
miR-582-3p	UAACUGGUUGAACAACUGA
	TTTTTTTTTTTTTTTTTTTTTTTTCCCCCCCCCTTT
hairpin H1	TTTTTTTTTTTTTTTTTTTTGTTGAACAACTGAC
	AAAGTTCAGTTGTTCAACCAGTTA
	ACTTTGTCAGTTGTTCAACTAACTGGTTGAACAA
hairpin H2	CTGATTTTTTTTTTTTTTTTTTTTTTTCCCCCCCC
	CTTTTTTTTTTTTTTTTTTTTTTT
miR-582-3p mimic	F: UAACUGGUUGAACAACUGA
	R: AGUUGUUCAACCAGUUAUU
SM miR-582-3p	UAACUAGUUGAACAACUGA
TM miR-582-3p	UAACUAGUUGACCACCUGA
NC-miRNA	UUGUACUACACAAAAGUAC
miR-21	UAGCUUAUCAGACUGAUGUUGA
miR-141	UAACACUGUCUGGUAAAGAUGG

F, forward; R, reverse; SM, single-base mismatched; TM, three-base mismatched; NC, nonspecific control. The red shown in sequences reveal the mismatch part.

Table S2. The analysis results of miR-582-3p content in cell lysates (n=3).

Sample	Found (nmol L^{-1})	Spiked (nmol L^{-1})	Recovery (%)
Lysate 1*	12.0±2.5	5	93±13
		10	102±12
		20	91±6
Lysate 2*	20.5±2.0	5	103±17
		10	90±5
		20	106±5

* The amount of the cells in lysate 1 and 2 were 2.10×10^6 and 2.65×10^6, respectively.

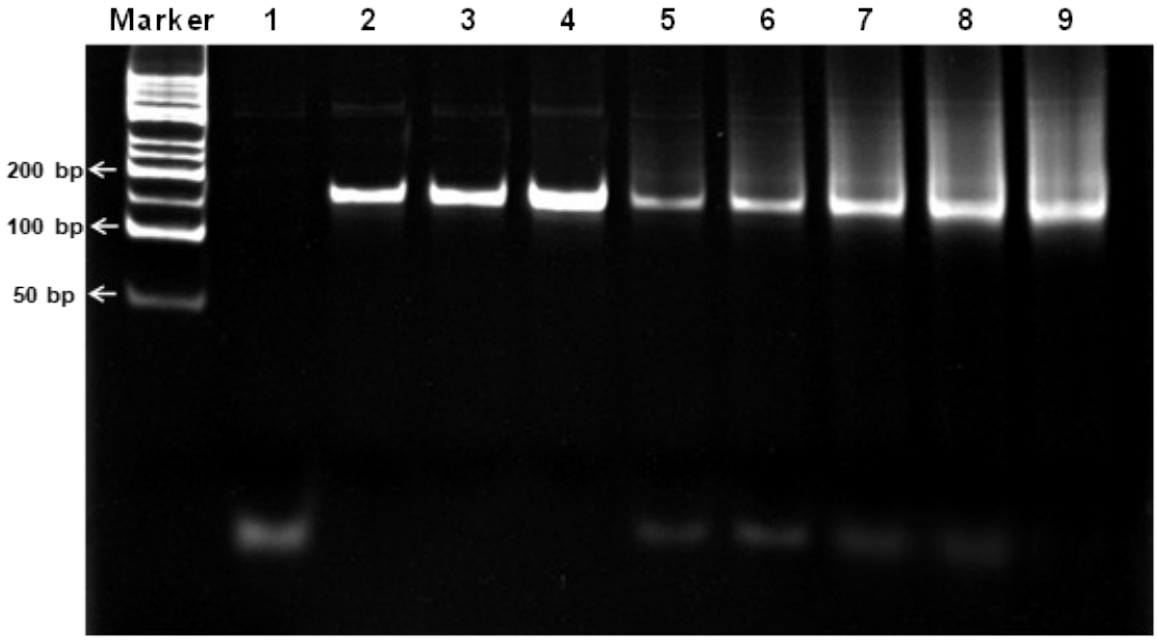

Figure S1. PAGE (12%) gel electrophoresis analysis of HCR reaction. Lane 1, target miRNA (1.2 μmol L^{-1}); lane 2, H1-CuNCs (1.2 μmol L^{-1}); lane 3, H2-CuNCs (1.2 μmol L^{-1}); lane 4, 1.2 μmol L^{-1} H1-CuNCs mixed with 1.2 μmol L^{-1} H2-CuNCs; lane 5-9, the HCR products. The concentrations of H1-CuNCs and H2-CuNCs used from lane 5 to 9 are 0.4, 0.6, 1.2, 1.8 and 2.4 μmol L^{-1}, respectively, with 1.2 μmol L^{-1} target miRNA. The gel was run at 180 V for 30 min in TBE buffer.

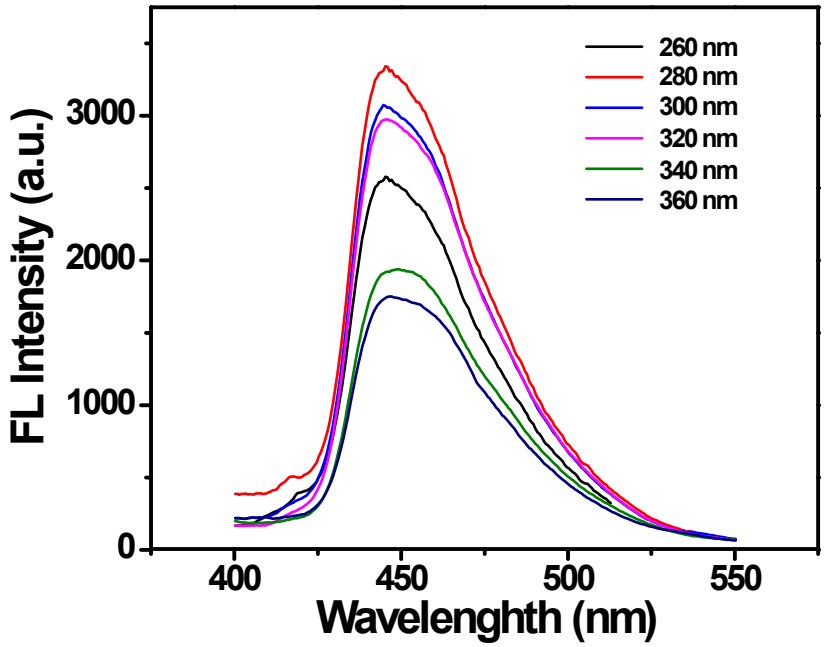

Figure S2. The emission spectra of BCNNS (100 μg mL^{-1}) at various excitation wavelengths.

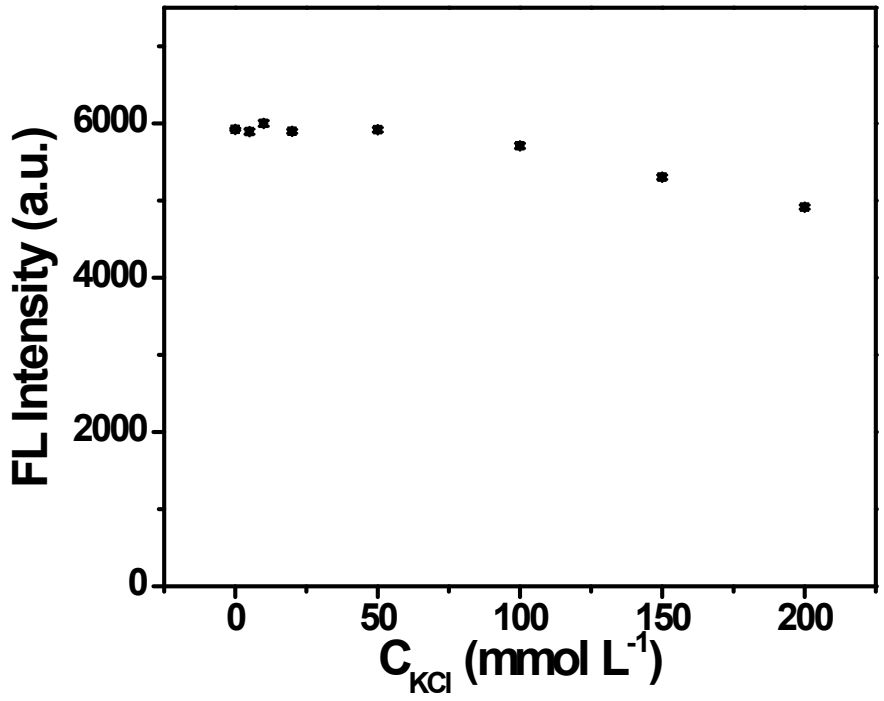

Figure S3. The variation of fluorescence intensity of CuNCs (100 nmol L^{-1}) as a function of ionic strength with excitation at λ_{ex} 340 nm.

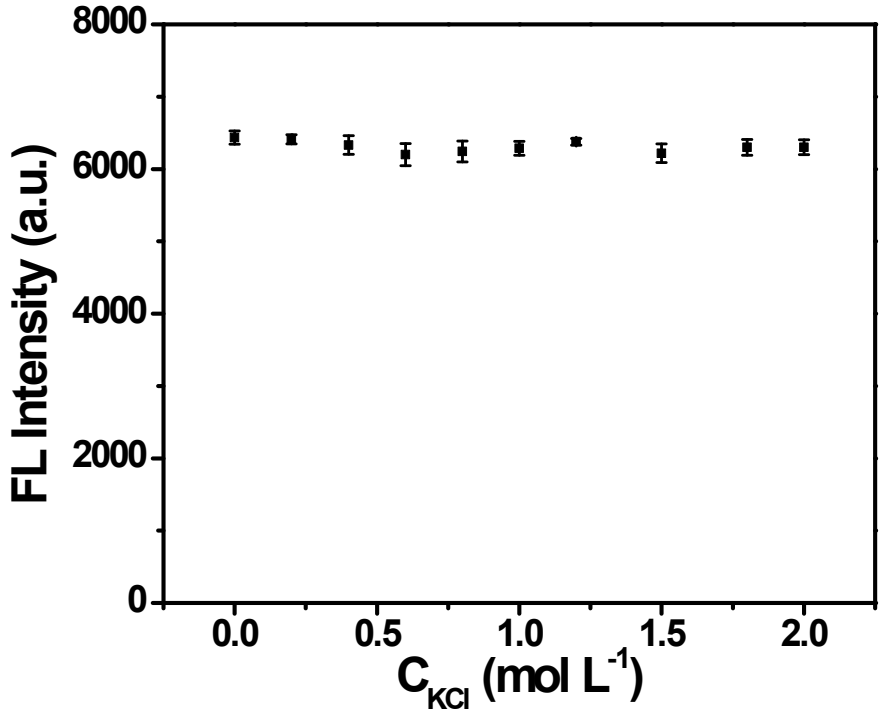

Figure S4. The variation of fluorescence intensity of BCNNS (100 μg mL^{-1}) as a function of ionic strength with excitation at λ_{ex} 340 nm.

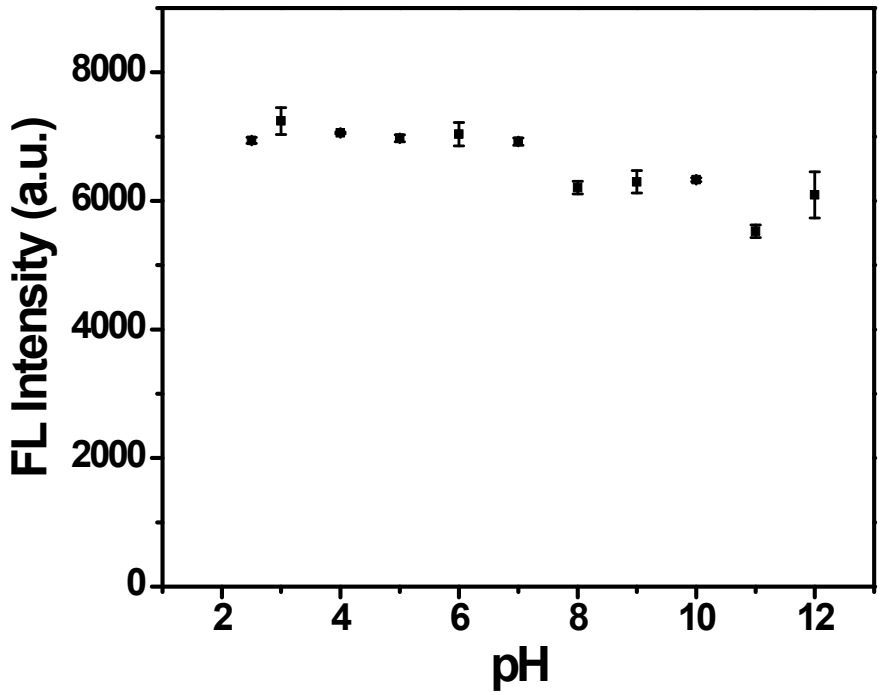

Figure S5. The variation of fluorescence intensity of BCNNS (100 μg mL^{-1}) as a function of pH value with excitation at λ_{ex} 340 nm.

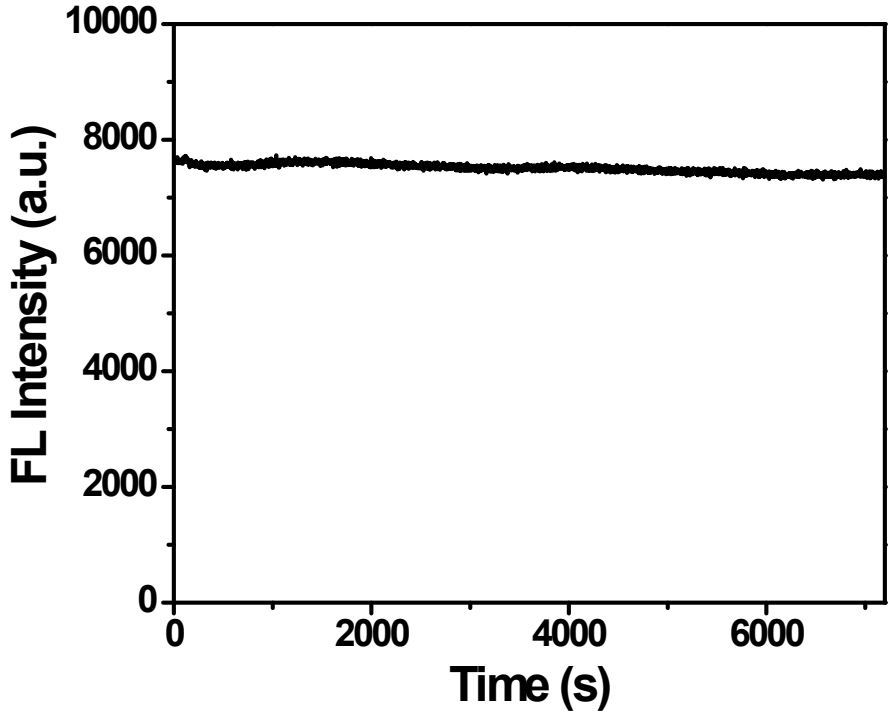

Figure S6. The variation of fluorescence intensity of BCNNS (100 μg mL^{-1}) as a function of irradiation time with excitation at λ_{ex} 340 nm.

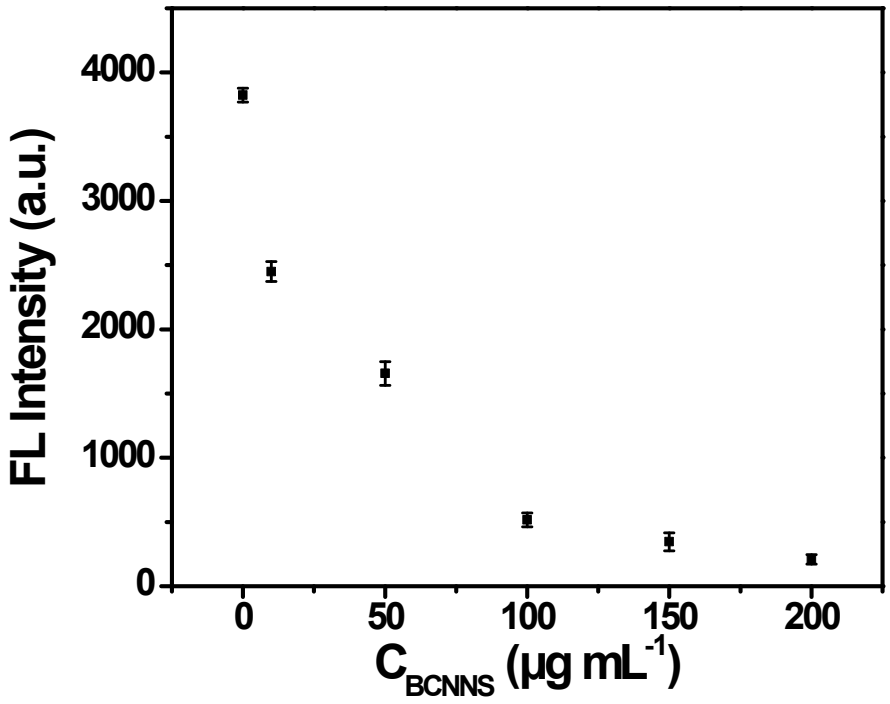

Figure S7. Quenching effect of BCNNS at various concentrations on CuNCs. The concentration of CuNCs is 100 nmol L^{-1}. BCNNS concentrations are given within a range of 0-200 μg mL^{-1}. The excitation and emission wavelengths are set at 340 and 600 nm, respectively.

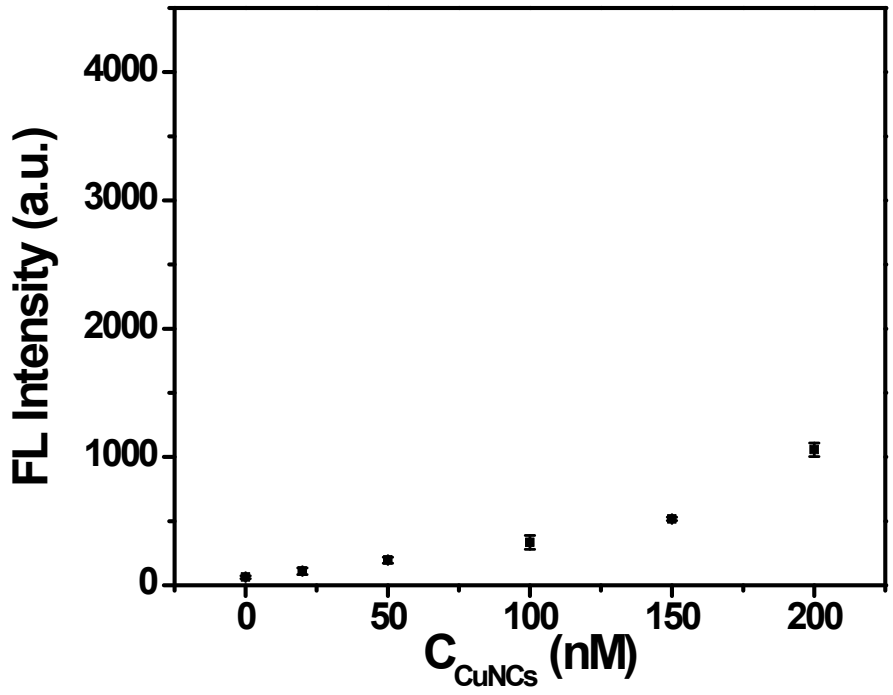

Figure S8. Quenching effect of BCNNS on the fluorescence of CuNCs with various concentrations. The concentration of BCNNS is 200 μg mL^{-1}. CuNCs concentrations are given in a range of 0-200 nmol L^{-1}. The excitation and emission wavelengths are set at 340 and 600 nm, respectively.

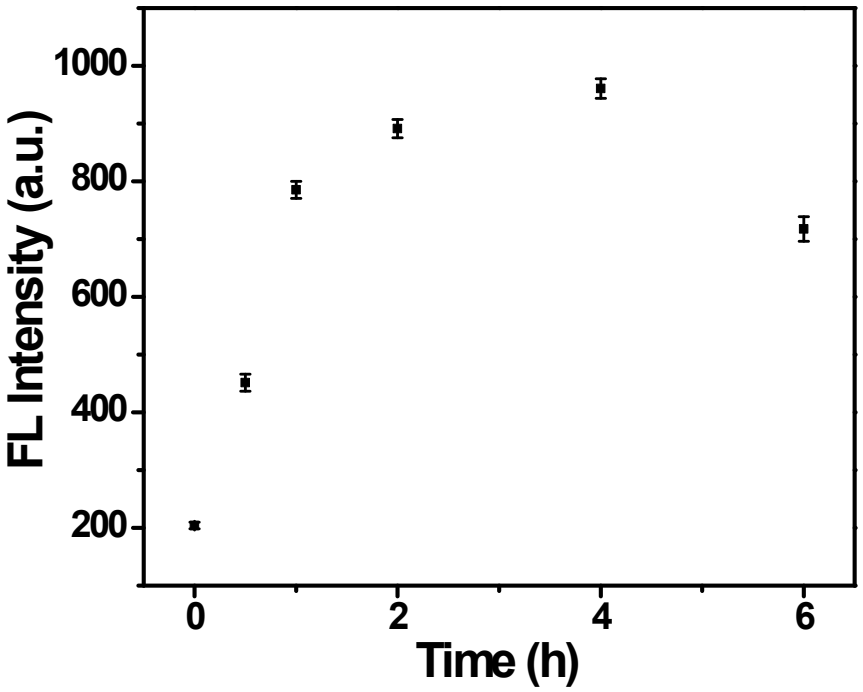

Figure S9. Time-dependent fluorescence changes of BCNNS-CuNCs in the presence of miR-582-3p (10 pmol L^{-1}). The excitation and emission wavelengths are set at 340 and 600 nm, respectively.

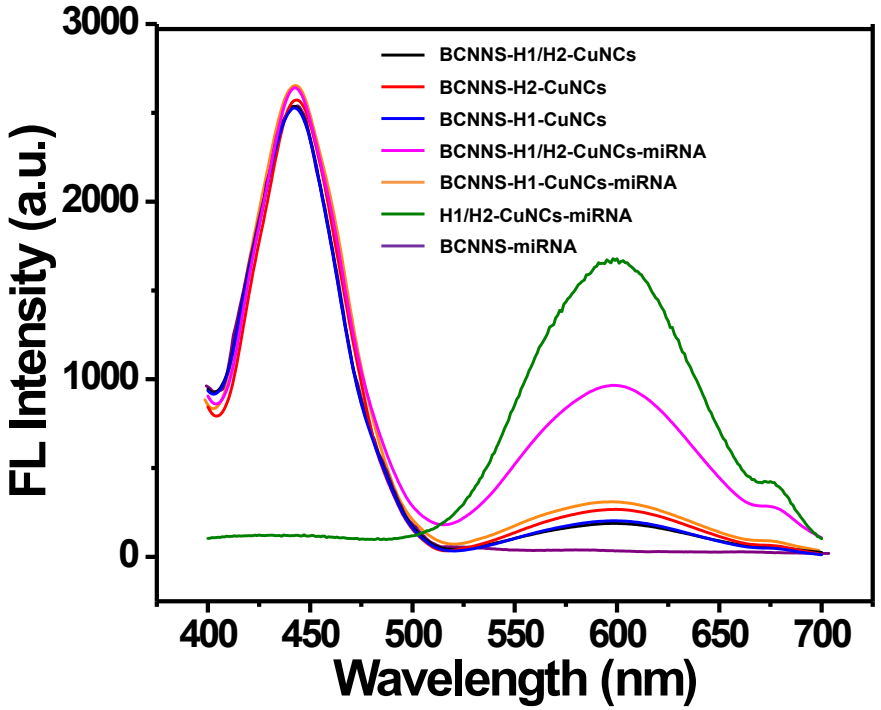

Figure S10. The fluorescence response of the system in the presence of miR-582-3p (10 pmol L^{-1}) under different conditions: BCNNS-H1/H2-CuNCs; BCNNS-H1-CuNCs; BCNNS-H2-CuNCs; BCNNS-H1/H2-CuNCs-miR-582-3p; BCNNS-H1-CuNCs-miR-582-3p; H1/H2-CuNCs-miR-582-3p and BCNNS-miR-582-3p. The concentrations of H1-CuNCs and H2-CuNCs are 100 nmol L^{-1}; the concentration of BCNNS is 200 μg mL^{-1}.

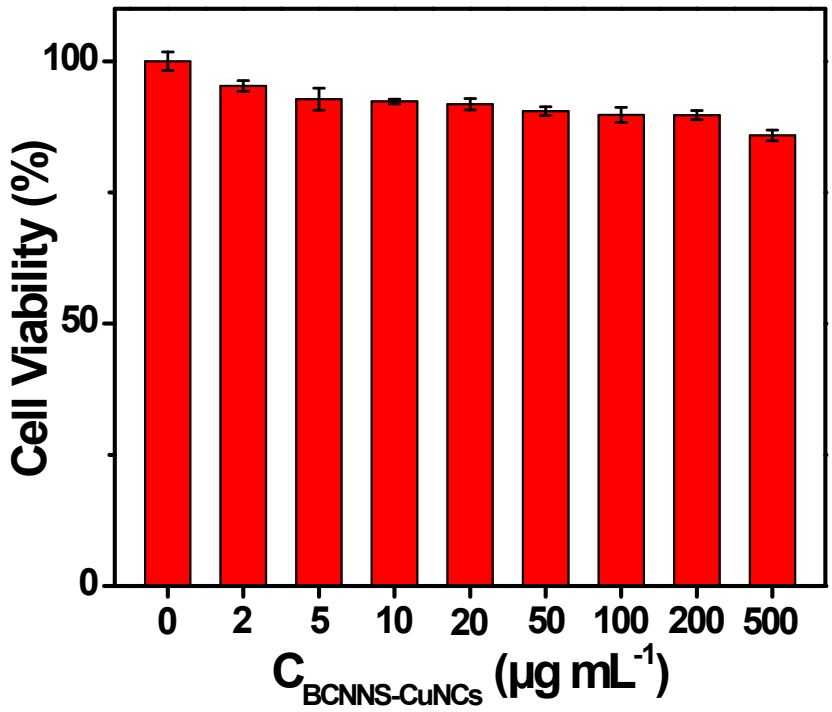

Figure S11. Effect of the BCNNS-CuNCs concentration (0, 2, 5, 10, 20, 50, 100, 200 and 500 μg mL^{-1}) on the viability of A549 cells with an incubation time of 24 h.

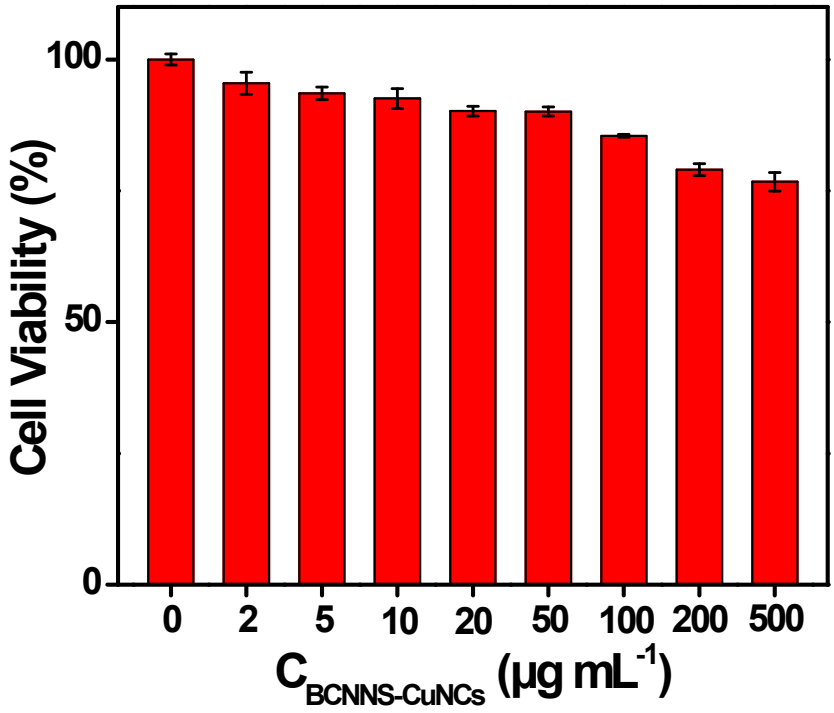

Figure S12. Effect of the BCNNS-CuNCs concentration (0, 2, 5, 10, 20, 50, 100, 200 and 500 μg mL^{-1}) on the viability of BEAS-2B cells with an incubation time of 24 h.

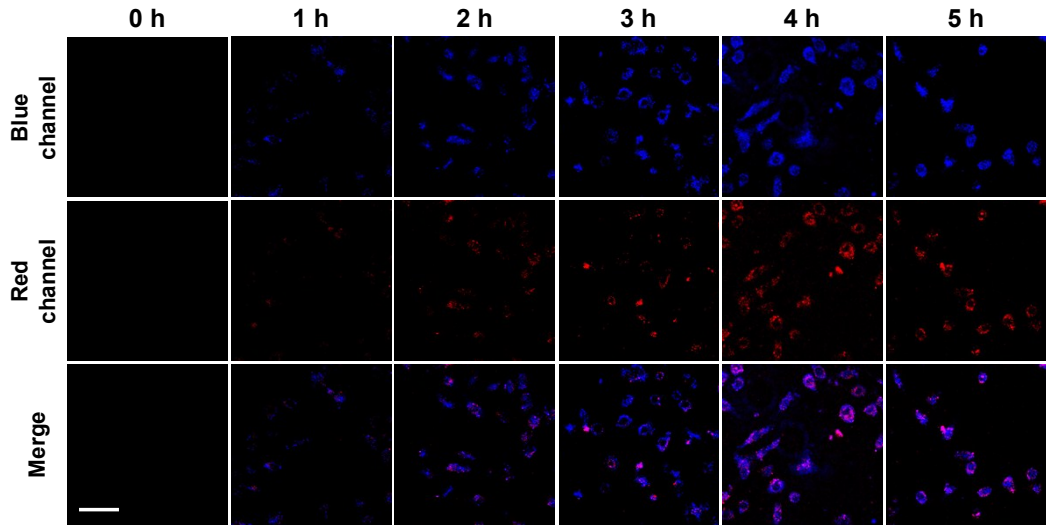

Figure S13. Confocal images of A549 cells incubated with BCNNS-CuNCs (200 μg mL^{-1}) for 0, 1, 2, 3, 4 and 5 h. Scale bar: 40 μm.

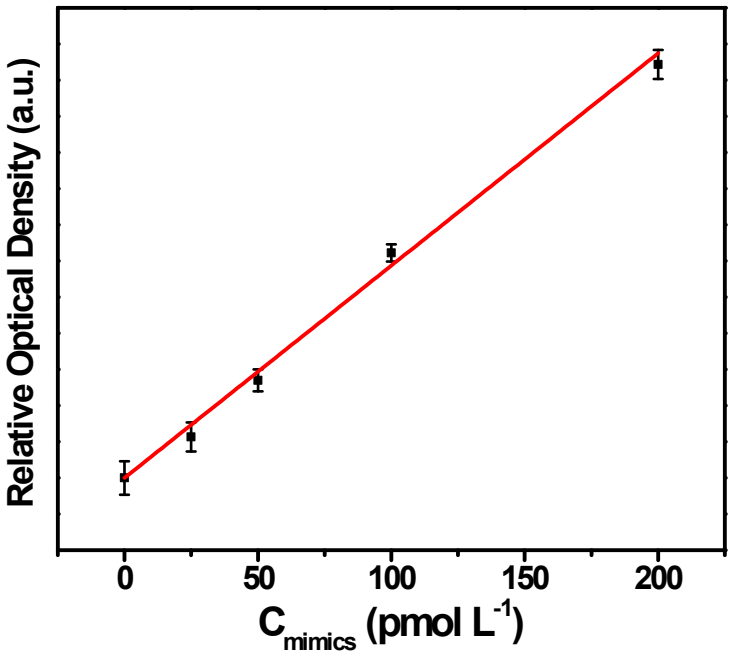

Figure S14. The calibration curve between miR-582-3p mimics concentration and relative optical density (red channel *vs* blue channel).