Electronic Supplementary Information for:

Malachite Green: A long-buried water-soluble AIEgen with near-infrared fluorescence for living cell nucleus staining

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1. Reagents and Instruments

Malachite Green (MG) was purchased from Acmec Co., Ltd. and were used as received. Cell counting kit-8 (CCK-8) were purchased from Beyotime (Shanghai, China). Unless stated otherwise, all commercial reagents used in the experiments were analytical pure and used directly without further purification.

The ^{1}H NMR spectra was recorded on a Bruker AVANCE III 400 in MeOD. ^{1}H NMR chemical shifts are reported in ppm relative to tetramethylsilane (TMS) with the solvent resonance employed as the internal standard (MeOD at 3.31 ppm). Data are reported as follows: chemical shift, multiplicity (s = singlet, br s = broad singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constants (Hz) and integration. High resolution mass spectra (HRMS) data were recorded on Thermo QExactive Focus. The fluorescence spectra were collected on an Edinburgh FLS 920 spectrophotometer. The absorption spectra were obtained on a Shimadzu UV-2600i spectrophotometer. Confocal laser scanning microscopy (CLSM) images were performed on a Leica (STELLARIS 5) confocal fluorescence microscope. The fluorescence imaging of **MG** power was performed on an IVIS Spectrum System. All the images were analyzed using ImageJ.

2. Theoretical Calculations

Density functional theory (DFT) calculations were employed to understand the electronic excitation and photophysical properties of **MG**. Geometry optimizations in the ground were carried out with B3LYP function in combination with the 6-31G(d) basis set. All calculations were carried out with Gaussian 16W.

3. Cell Culture and Cytotoxicity Test

The HeLa cells, 4T1 cells, Neuron cells and 293T cells were cultured in

Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) in a 5% CO2 atmosphere at 37 °C. Then, cells were incubated with **MG** at different concentrations of (0, 3, 6, 12 and 25 μM) for 12 h. Then removed the old medium and washed the wells three times with PBS buffer. Subsequently, 100 μL fresh medium and 10 μL of CCK-8 was added and incubated for another 1.5 h. Finally, the absorbance was recorded at 450 nm using a Tecan Infinite M Plex microplate reader.

4. Characterization Data

^{1}H NMR (400 MHz, MeOD) δ (ppm) 7.79 - 7.70 (m, 1H), 7.61 (t, J = 7.8 Hz, 2H), 7.49 -7.35 (m, 6H), 7.11 - 7.02 (m, 4H), 3.34 (s, 12H).

HRMS (ESI) calcd. for $C_{23}H_{25}N_2$ $[M]^+$ 329.20123, found: 329.20102.

5. Supplemental Figures

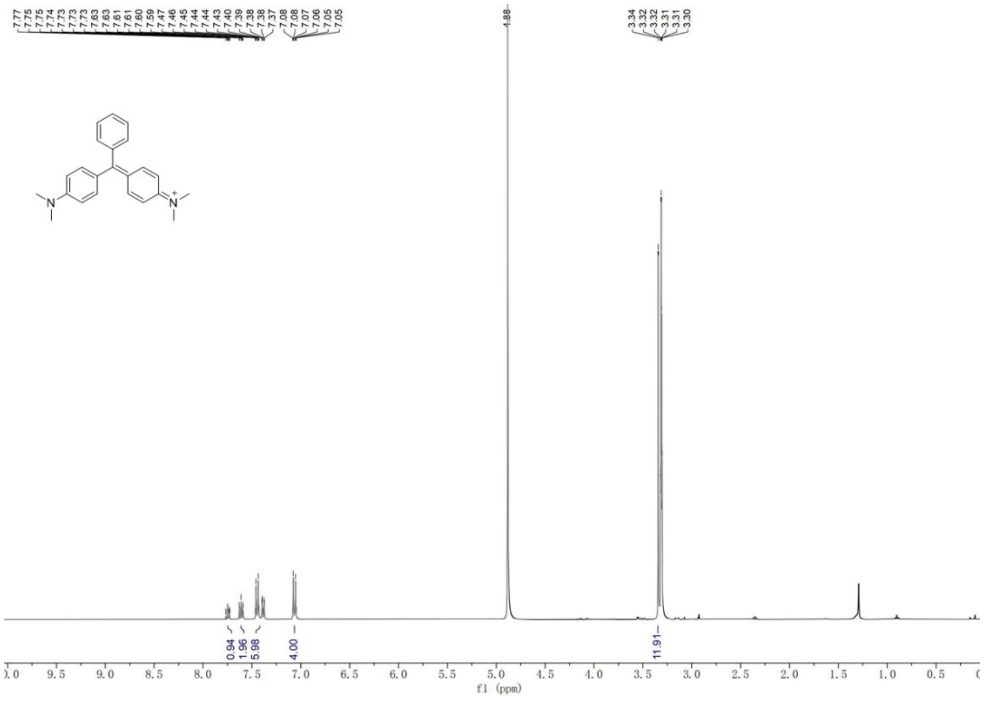

Figure S1. ^{1}H NMR spectrum (400 MHz) of Malachite Green **(MG)** in MeOD.

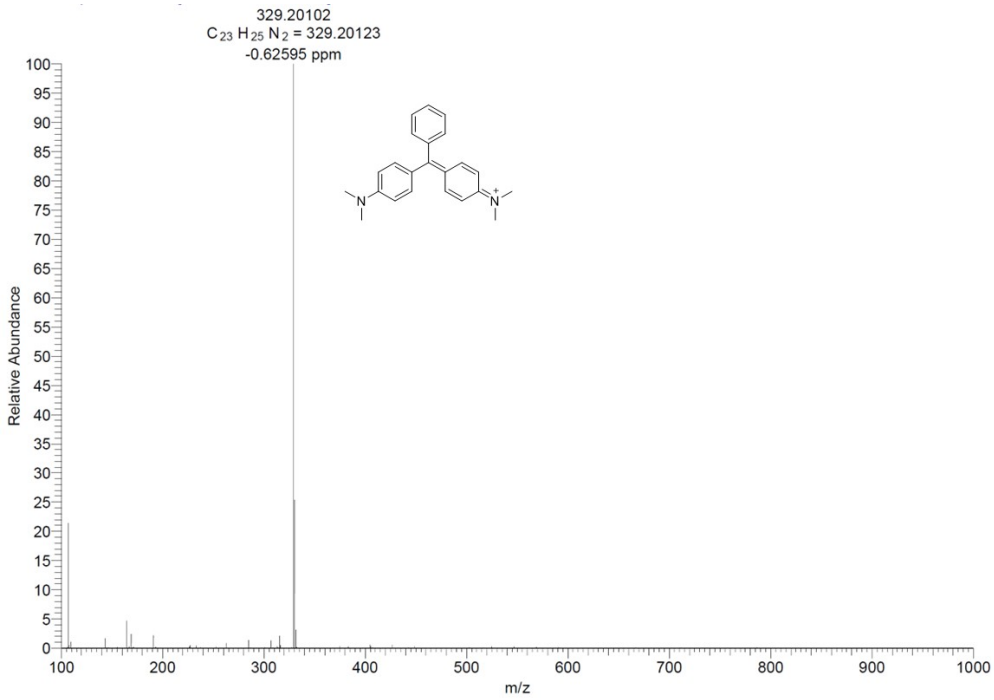

Figure S2. HRMS (ESI) of Malachite Green **(MG)**.

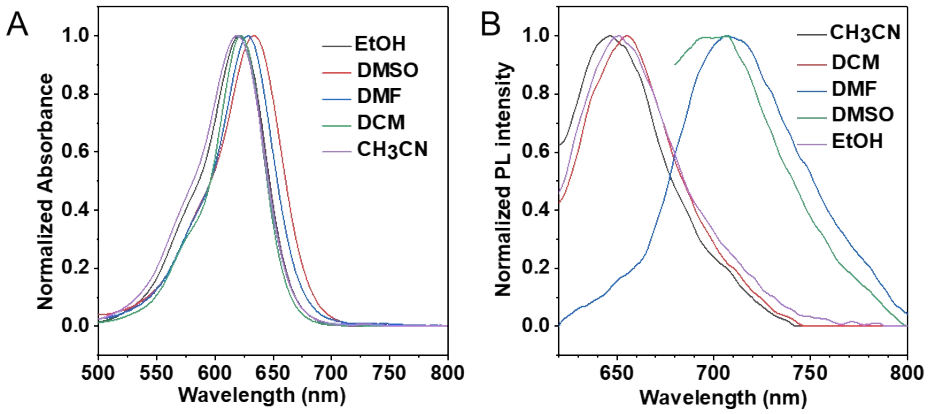

Figure S3. Normalized absorption (A) and PL spectra (B) of **MG** in different solvents (DMSO: dimethyl sulfoxide, DMF: dimethylformamide, DCM: dichloromethane).

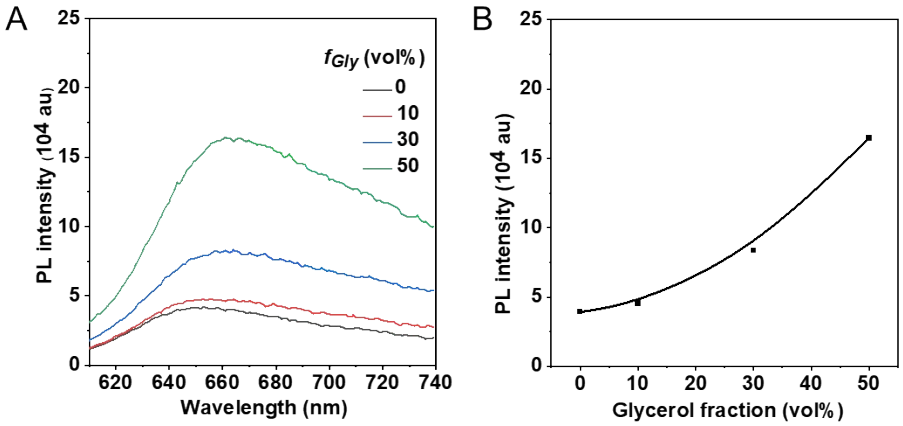

Fig. S4 (A) PL spectra of **MG** in water/glycerol mixtures with different glycerol fractions (f_{Gly}). (B) Plotting of the emission maximum of **MG** versus the fraction of the H_2O/Glycerol mixtures.

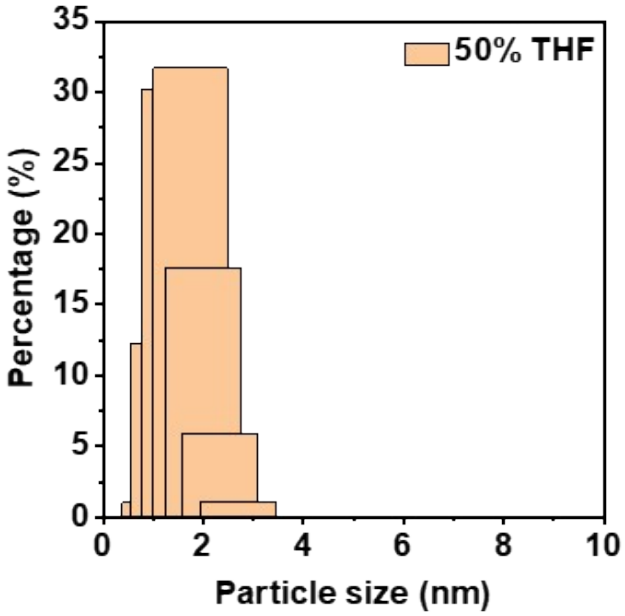

Fig. S5 The DLS measurement result of Malachite Green in the mixture of H_2O/THF with THF fraction of 50 vol%.

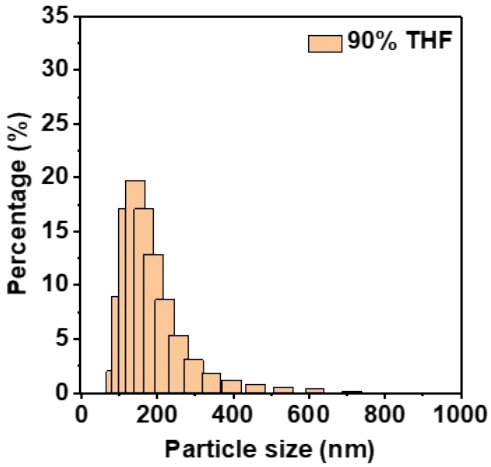

Fig S6. The DLS measurement result of Malachite Green in the mixture of H_2O/THF with THF fraction of 90 vol%.

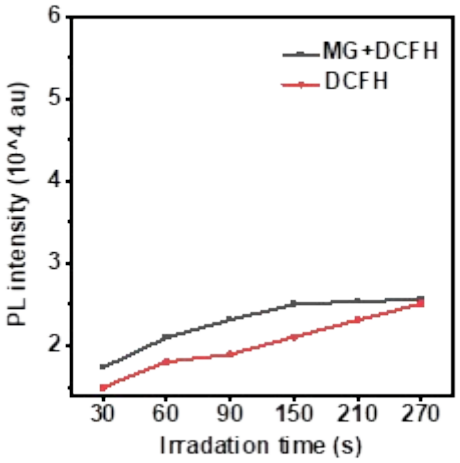

Fig. S7 ROS generation of **MG** upon white light irradiation. Relative changes in PL intensity of DCFH (for overall ROS detection)

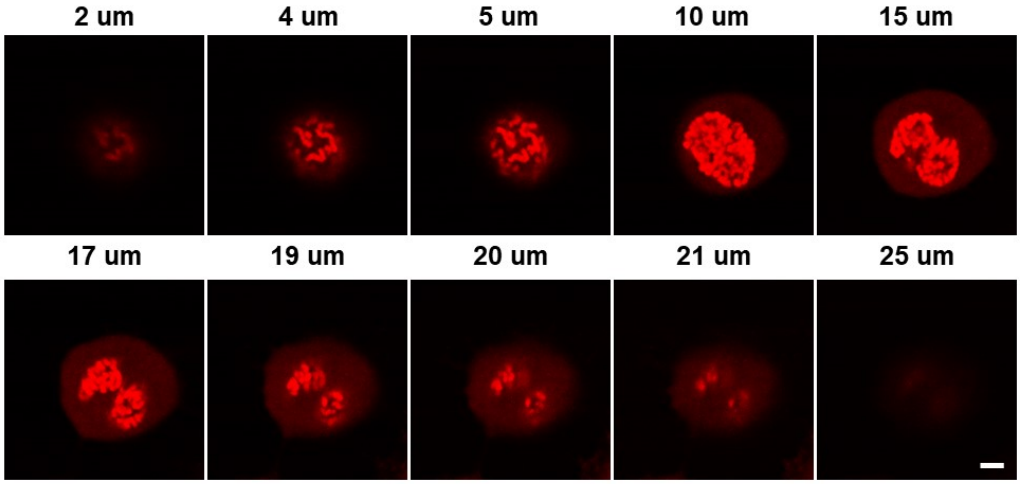

Fig. S8 Z-axis scanning of **MG**-stained Hela cell nuclei revealed through confocal microscopy. Hela cells were cultured on glass bottom dishes for 24 h before observation, and then the culture medium was replaced with SFM mixed with **MG** and cocultured for 5 min. Micrographs were taken while moving the focal plane in incremental steps from the dish bottom up to the top of the cell. The fluorescence images are shown here. Scale bar = 5 μm.

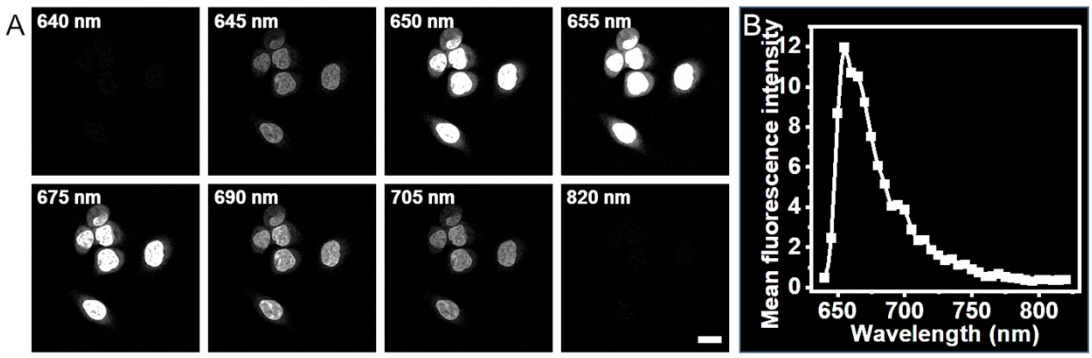

Fig. S9 Spectral imaging. (A) Spectral images of HeLa cells stained by **MG**. Scale bar = 10 μm. (B) Spectra of the HeLa cells.

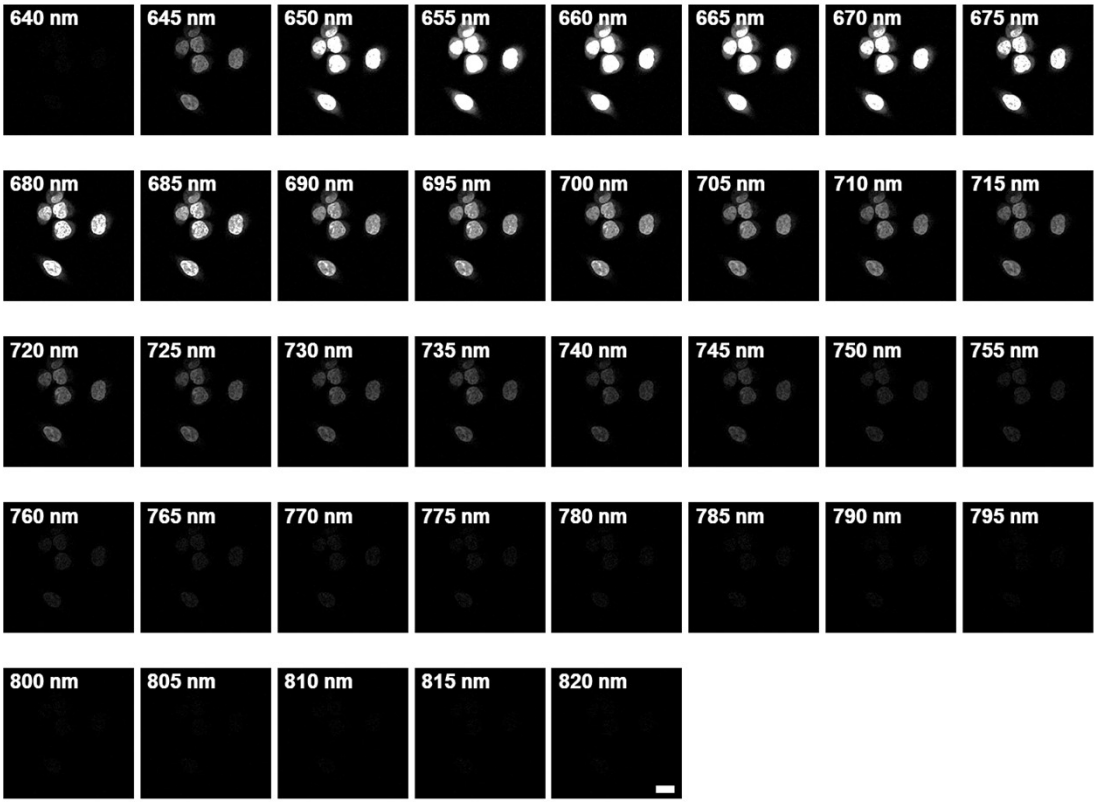

Fig. S10 Spectral images of HeLa cells. Scale bar = 10 μm.

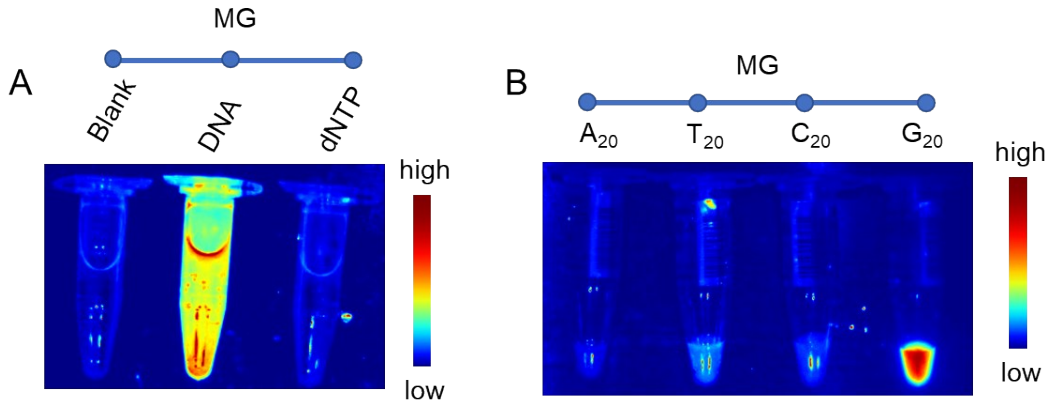

Fig. S11 (A) Fluorescence photo of the mixture of Malachite Green incubated with DNA or dNTP under 640nm light excitation. (B) Fluorescence photo of the mixture of Malachite Green incubated with DNA with our 20-mer DNA homopolymers (A_{20}=poly A, T_{20}=poly T, C_{20}=poly C, G_{20}=poly G) under 640nm light excitation.

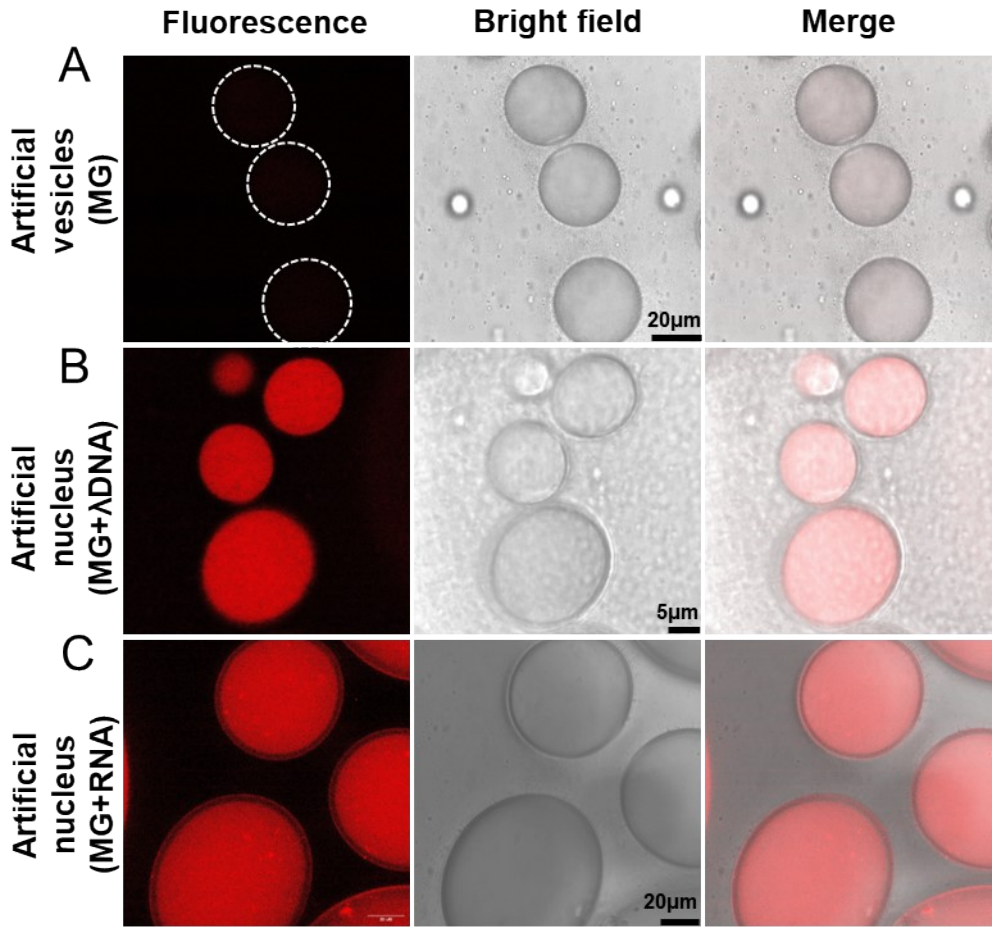

Fig. S12 Confocal images of droplets generated by microfluidic devices: (A) Artificial vesicles (**MG**), Scale bar = 20 μm. (B) Artificial nucleus (**MG**+λDNA), Scale bar = 5 μm. (C) Artificial nucleus (**MG**+RNA), Scale bar = 20 μm.

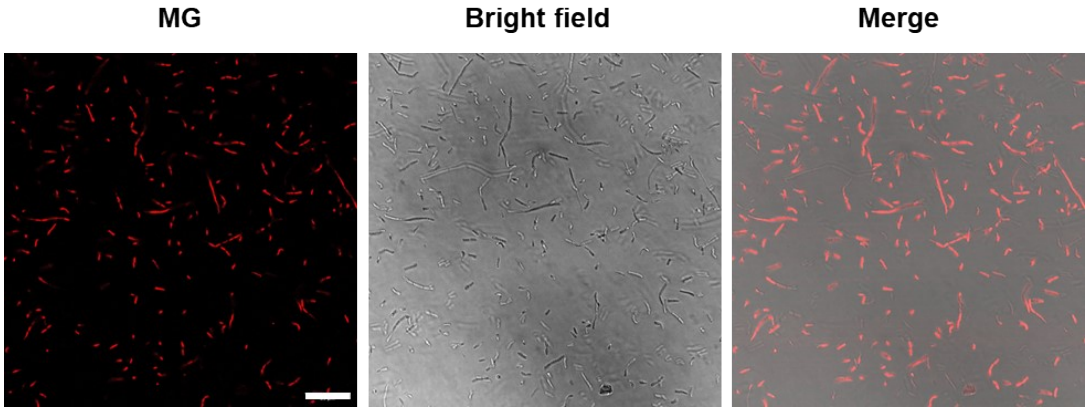

Fig. S13 Colocalization images of marine Bacillus sp. BS11 stained with **MG**. **MG** (λ_{ex} = 638 nm, λ_{em} = 650-850 nm). Scale bar = 20 μm.

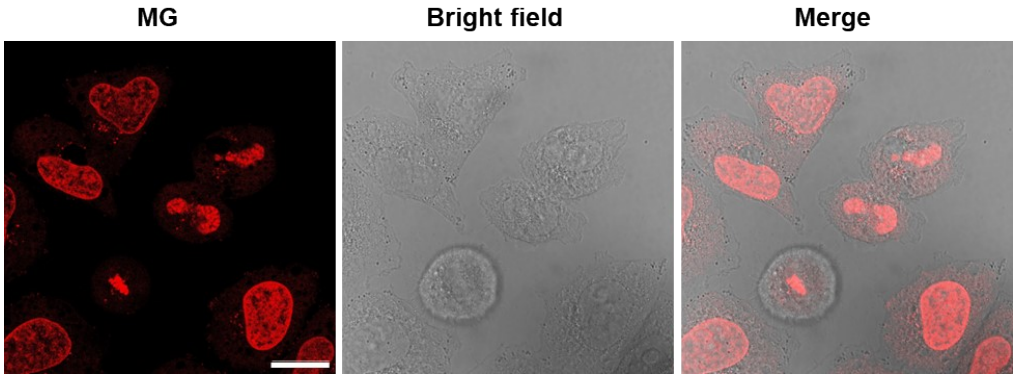

Fig. S14 Colocalization images of fixed HeLa cells with **MG**. **MG** (λ_{ex} = 638 nm, λ_{em} = 650-850 nm). Scale bar = 20 μm.

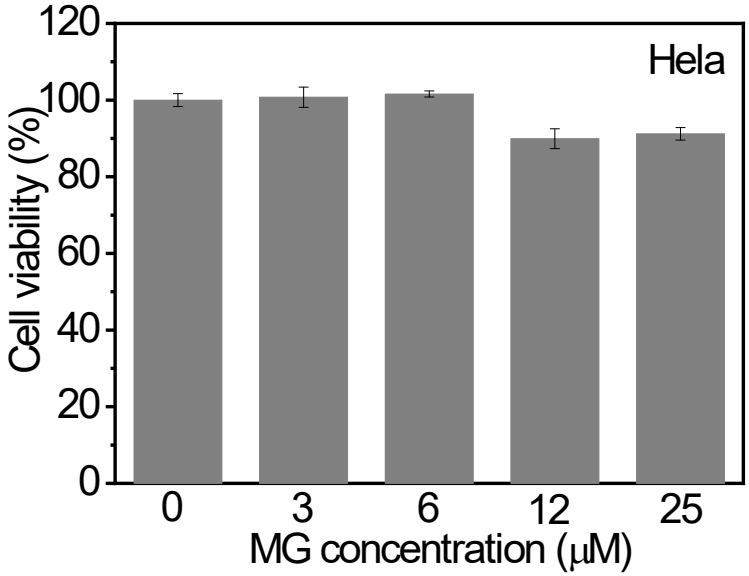

Fig. S15 The cell viability of HeLa after incubating with different concentrations of **MG** after 0.5 h by CCK-8 assay.

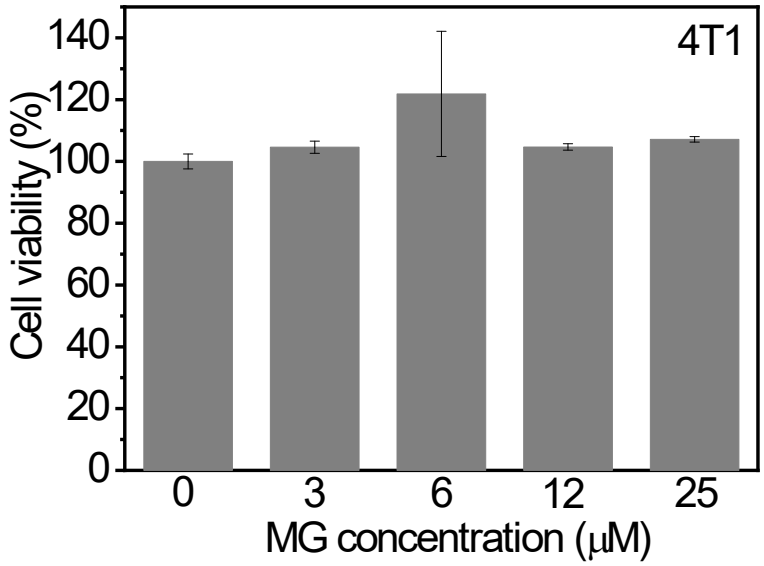

Fig. S16 The cell viability of 4T1 after incubating with different concentrations of **MG** after 0.5 h by CCK-8 assay.

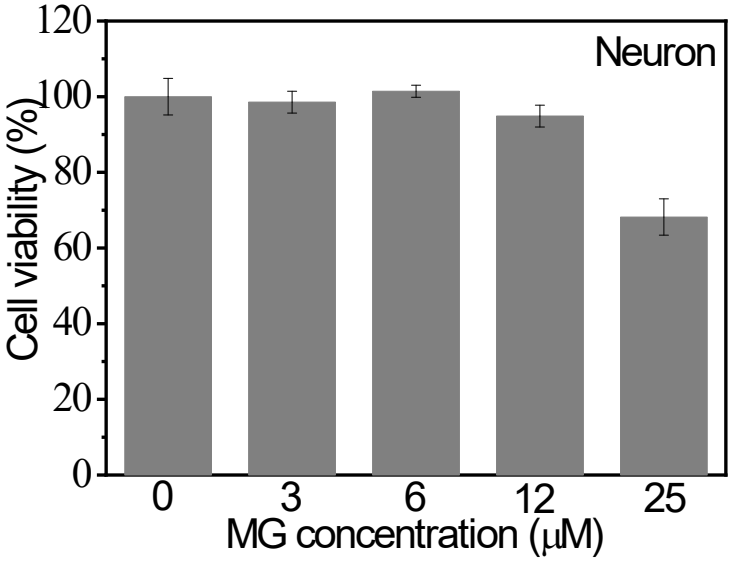

Fig. S17 The cell viability of neuron after incubating with different concentrations of **MG** after 0.5 h by CCK-8 assay.

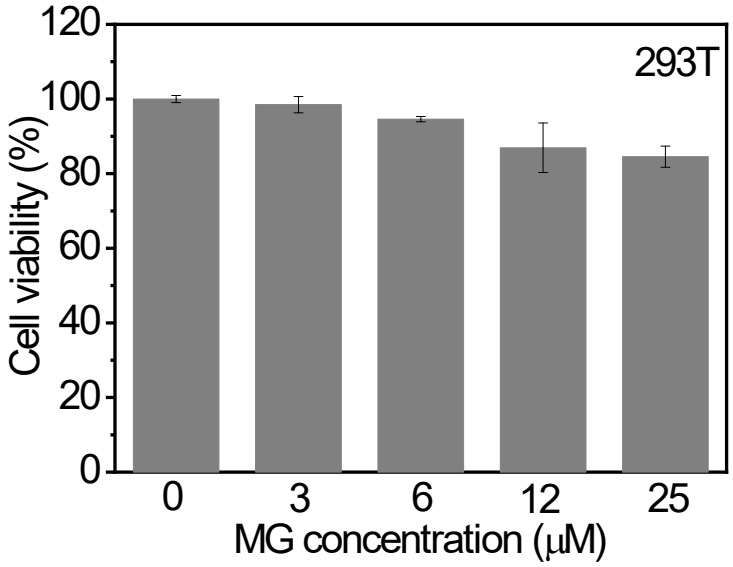

Fig. S18 The cell viability of 293T after incubating with different concentrations of **MG** after 0.5 h by CCK-8 assay.

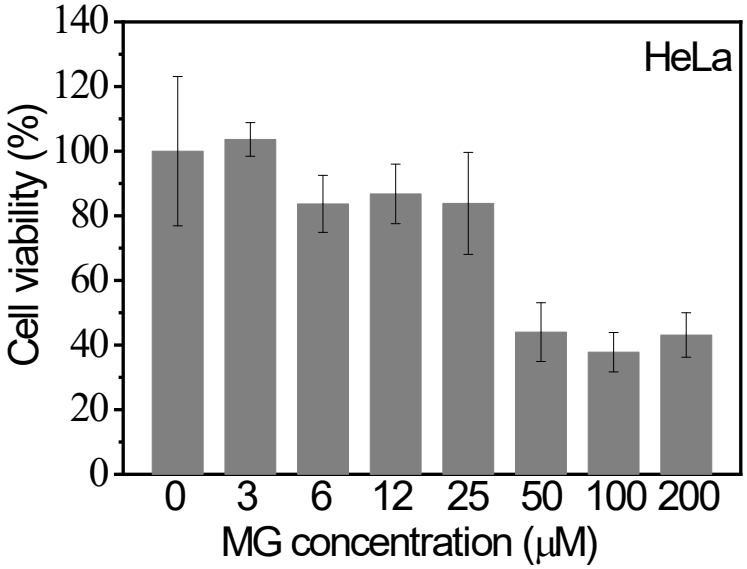

Fig. S19 The cell viability of HeLa after incubating with different concentrations of **MG** after 24 h by CCK-8 assay.

Table S1. The comparison of exist nucleus staining fluorescent probe.

References	Name of the Probes	Em.(nm)	Live cells	Fixed cells
Anal.Chem.2017, 89, 78	PDA	465	yes	no
Sci Rep, 2016, **6**, 34807	1	351/448	yes	no
Biomaterials, 2014, **35**, 2103	PY and IN	530	yes	no
ACS Sens, 2021, **6**, 1552	HMBI	550/605	yes	yes
Photochem Photobiol Sci, 2020, **19**, 1152	1	604	yes	no
Sensors and Actuators B: Chemical, 2021, **347**	LN-2	525	no	yes
Biosens Bioelectron, 2015, **68**, 189	CP	658	yes	no
Anal Chim Acta, 2020, **1096**, 148	RatioTr	545	yes	no
Chem Commun, 2015, **51**, 9336	CDb12	490	yes	no
ACS Chem Biol, 2015, **10**, 1171	MPI	536	yes	no
Sci Rep, 2016, **6**, 26477	CZtpyZn	570	yes	no
This work	MG	696	yes	yes
Commercial reagents	Name of the Probes	Em.(nm)	Live cells	Fixed cells
Thermo Fisher Scientific Inc.	Hoechst 33342	461	yes	no
Thermo Fisher Scientific Inc.	SYTO™ 9 Green Fluorescent Nucleic Acid Stain	RNA: 501 nm DNA: 498 nm	yes	no
Thermo Fisher Scientific Inc.	SYTO™ 82 Orange Fluorescent Nucleic Acid Stain	560	yes	no
Thermo Fisher Scientific Inc.	SYTO™ 59 Red Fluorescent Nucleic Acid Stain	645	yes	yes
Thermo Fisher Scientific Inc.	SYTO™ Deep Red Nucleic Acid Stain	669	yes	no
Thermo Fisher Scientific Inc.	DAPI	460	no	yes
Thermo Fisher Scientific Inc.	SYTOX™ Blue Nucleic Acid Stain	480	no	yes
Thermo Fisher Scientific Inc.	SYTOX™ Green Nucleic Acid Stain	523	no	yes
Thermo Fisher Scientific Inc.	SYTOX™ Orange Nucleic Acid Stain	570	no	yes
Thermo Fisher Scientific Inc.	SYTOX™ Deep Red Nucleic Acid Stain	682	no	yes
Thermo Fisher Scientific Inc.	TO-PRO	661	no	yes