### **Supporting Information**

#### Light-up Endoplasmic Reticulum Probe based on Rational Design of

#### **Red-emissive Fluorogens with Aggregation-Induced Emission**

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**General Information** 9,10-Anthracenediyl-bis(methylene)dimalonic acid (ABDA), 3-(4,5-dimethyl- thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and other chemicals were all purchased from Sigma-Aldrich and used as received without further purification. Compound **NTPE-DCV**, **1** and **2** were synthesized according to previous published procedure[1]. Dry dichloromethane and dimethylformamide (DMF) were distilled over CaH<sub>2</sub>. All non-aqueous reactions were carried out in ovendried glassware under nitrogen atmosphere. High-performance liquid chromatography (HPLC) was performed in Agilent technologies (1200 series) with acetonitrile containing trifluoroacetic acid (0.1%) and water containing trifluoroacetic acid (0.1%) as the elution buffer. NMR spectra were recorded on a Bruker ARX 400/500 NMR spectrometer. Chemical shifts were recorded in parts per million referenced according to residual solvent ((CD<sub>3</sub>)<sub>2</sub>SO = 2.50 ppm and CDCl<sub>3</sub> = 7.26 ppm) in <sup>1</sup>H NMR and ((CD<sub>3</sub>)<sub>2</sub>SO = 40.0 ppm and CDCl<sub>3</sub> = 77.0 ppm) in <sup>13</sup>C NMR. Mass spectra were reported on the AmaZon X LC-MS for ESI.

#### 1 Synthesis of 3 and 4



To the solution of 2-cyanoacetate (100 mg, 1.0 mmol) in IPA (5 mL) was added 2 (100 mg, 0.22 mmol) in DCM (1 mL). After piperidine (20  $\mu$ L, 0.20 mmol) was added to the above mixture, the reaction mixture was stirred at room temperature overnight. The solvent was removed under reduced pressure and the residue was purified with chromatography (hexane/ethyl acetate = 10:1-3:1) to yield the main product (3) as a red solid (30 mg), and a side product (4) as a red solid (6.0 mg).

**3**: <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>CN) δ 8.17 (s, 1H), 7.76 (d, *J* = 8.4 Hz, 2H), 7.16 (m, 5H), 7.05 (m, 2H), 6.83-6.87 (m, 4H), 6.46-6.51 (m, 4H), 3.86 (s, 3H); 2.88 (brs, 12H); <sup>13</sup>C NMR (100 MHz, CD<sub>3</sub>CN) δ 164.0, 155.2, 150.6, 150.4, 133.4, 133.2, 133.0, 132.3, 131.4, 129.7, 128.8, 127.1, 116.8, 112.3, 112.0, 102.1, 53.8, 40.4; HRMS (ESI), calcd for [M+Na]<sup>+</sup>: 550.2465, found: 550.2469.



4: <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>) δ 8.20 (s, 1H), 7.81 (d, *J* = 7.2 Hz, 2H), 7.07-7.17 (m, 6H), 6.97 (m, 2H), 6.79 (m, 4H), 6.43-6.49 (m, 4H), 5.10 (m, 1H), 2.85 (s, 6H), 2.83 (m, 6H), 1.30 (d, *J* = 4.8 Hz, 6H); HRMS (ESI), calcd for [M+Na]<sup>+</sup>: 578.2778, found: 578.2783.

#### 2 Synthesis of 5 and 6



To the solution of 2-cyano-N,N-diethylacetamide (76 mg, 0.54 mmol) in IPA (5 mL) was added 2 (100 mg, 0.22 mmol) in DCM (1 mL). After piperidine (20  $\mu$ L, 0.20 mmol) was added to the above mixture, the reaction mixture was stirred at 60 °C overnight. Then the reaction mixture was cooled down to room temperature. The solvent was removed under reduced pressure and the residue was purified with chromatography (hexane/ethyl acetate = 10:1-3:1) to yield the major product in *trans* form as a red solid (30 mg), and a minor product in *cis* form as a red solid (6.0 mg). *Trans* form (**5**): <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>CN)  $\delta$  7.64 (d, *J* = 8.0 Hz, 2H), 7.43 (s, 1H), 7.11-7.17 (m, 5H), 7.03-7.05 (m, 2H), 6.83-6.87 (m, 4H), 6.46-6.51 (m, 4H), 3.46 (m, 4H), 2.87 (s, 6H), 2.86 (s, 6H), 1.20 (m, 6H); HRMS (ESI), calcd for [M+Na]<sup>+</sup>: 591.3094, found: 591.3099.



*Cis* form (6):<sup>1</sup>H NMR (400 MHz, DMSO- $d_6$ )  $\delta$  7.54 (s, 1H), 7.08-7.20 (m, 5H), 6.93-6.96 (m, 4H), 6.77 (dd,  $J_1 = 2.4$  Hz,  $J_2 = 8.8$  Hz), 6.49 (m, 4H), 3.36 (q, J = 7.2 Hz, 2H), 3.17 (q, J = 7.2 Hz, 2H), 0.98 (t, J = 7.2 Hz, 3H), 0.96 (t, J = 7.2 Hz, 3H); HRMS (ESI), calcd for [M+Na]<sup>+</sup>: 591.3094, found: 591.3100.

#### 3 Synthesis of 7

To the solution of 3-Methylamino-1-propanol (0.33g, 3.75 mmol) in dichloromethane (20 mL) was added triethylamine (0.6mL, 4.28 mmol) and di-*tert*-butyl dicarbonate (850 mg) in an ice-water bath. After 1 hour, the reaction mixture was stirred at room

temperature overnight. The mixture was washed with brine (15 mL $\times$ 2), NH<sub>4</sub>Cl (aq,

15 mL×2), NaHCO<sub>3</sub> (aq, 15 mL×2) and dried over Na<sub>2</sub>SO<sub>4</sub>. The mixture was filtered and the filtrate was condensed and purified with chromatography (hexane/ethyl acetate = 3:1) to yield the desired product as colorless oil (550 mg, 77.6%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  3.53 (m, 2H), 3.37 (m, 2H), 2.82 (s, 3H), 1.68 (m, 2H), 1.47 (s, 9H).

#### 4 Synthesis of 8

Boc N OTs (8)

To the solution of 7 (520 mg, 2.74 mmol) in pyridine (5 mL) was added 4toluenesulfonyl chloride (600 mg, 3.14 mmol) in an ice-water bath. The mixtue was stirred at same temperature for 4 hours. Then most of pyridine was removed under reduced pressure. To the residue was added ethyl acetate (50 mL) and water (30 mL). The organic phase was taken, washed with NH<sub>4</sub>Cl (aq, 15 mL×3), NaHCO<sub>3</sub> (aq, 15 mL) and brine (15 mL), and dried with Na<sub>2</sub>SO<sub>4</sub>. The mixture was filtered and the filtrate was condensed and purified with chromatography (hexane/ethyl acetate = 5:1) to yield the desired product as white solid (550 mg, 58.5%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.75 (m, 2H), 7.32 (m, 2H), 4.02 (t, *J* = 6.4 Hz, 2H), 3.22 (t, *J* = 6.8 Hz, 2H), 2.75 (s, 3H), 2.41 (s, 3H), 1.85 (m, 2H), 1.38 (s, 9H).

#### 5 Synthesis of 9



To the solution of **8** (550 mg, 1.60 mmol) in dry DMF (10 mL) was added 3a,4,7,7a-tetrahydro-1H-4,7-epoxyisoindole-1,3(2H)-dione (350 mg, 2.1 mmol) and K<sub>2</sub>CO<sub>3</sub> (550 mg, 4.0 mmol) at room teperature. Then the reaction mixture was stirred at 50 °C overnight. The mixture was cooled down to room temperature and then most of DMF was removed under reduced pressure. To the residue was added ethyl acetate (50 mL)

and water (30 mL). The organic phase was taken, washed with  $NH_4Cl$  (aq, 15 mL $\times$ 3), NaHCO<sub>3</sub> (ag, 15 mL) and brine (15 mL), and dried with Na<sub>2</sub>SO<sub>4</sub>. The mixture was filtered and the filtrate was condensed and purified with chromatography (hexane/ethyl acetate = 2:1) to yield the desired product as white solid (280 mg, 52.0%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  6.47 (m, 2H), 5.22 (m, 2H), 3.44 (t, J = 7.2 Hz, 2H), 3.16 (m, 2H), 2.80(s, 2H), 2.78 (s, 3H), 1.71-1.78 (m, 2H), 1.40 (s, 9H); <sup>13</sup>C NMR (100 MHz, CD<sub>3</sub>CN) δ 176.0, 136.4, 80.8, 47.3, 36.5, 34.0, 28.3.

#### 6 Synthesis of 10



To the solution of 9 (280 mg, 1.60 mmol) in dichloromethane (5 mL) was added TFA (1.5 mL) at room temperature. Then the mixture was stirred at the same temperature for 8 hours. The mixture was condensed under reduced pressure. The resulting residue was dissolved in methyl alchohol (3 mL). To this solution was added methyl 2cyanoacetate (300 mg, 3.0 mmol) and DBU (304 mg, 2.0 mmol). The resulitng mixture was stirred at room temperature for 3 days. The solvent was removed under reduced pressure. And the residue was purified with chromatography (hexane/ethyl acetate = 2:1) to give the product colorless oil (80 mg, 16.4%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  6.52 (m, 2H), 5.24-5.27 (m, 4H), 3.46-3.57 (m, 4H), 3.39 (t, J = 7.2 Hz, 1.3H), 3.24 (m, 0.8 H), 3.02 (s, 1.8 H), 2.92 (s, 1.2 H), 2.89 (s, 0.8 H), 2.87 (s, 1.2 H), 1.82-1.95 (m, 2H). HRMS (ESI), calcd for [M+Na]+: 326.1117, found: 326.1116.

#### 7 Synthesis of 2-cyano-N,N-diethylacetamide

NC (2-cyano-N,N-diethylacetamide) Diethyl amine (160 mg, 2.2 mmol) and 2-cyanoacetate (220 mg, 2.2 mmol) was mixed and stirred at room temperature for 3 days. Then the mixture was purified with chromatography (hexane/ethyl acetate = 5:1-2:1) to give the product as colorless oil (120 mg, 39.0%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 3.48 (s, 2H), 3.36 (q, J = 7.2 Hz, 2H), 3.27 (q, J = 7.2 Hz, 2H), 1.19 (t, J = 7.2 Hz, 3H), 1.10 (t, J = 7.2 Hz, 3H); 160.7, 114.2, 42.6, 40.7, 24.7, 13.8, 12.5.

#### 8 Synthesis of 11



To the solution of 10 (60 mg, 0.20 mmol) in IPA (5 mL) was added 2 (120 mg, 0.26 mmol) in DCM (2 mL). After piperidine (15  $\mu$ L, 0.15 mmol) was added to the above mixture, the reaction mixture was stirred at 50 °C overnight. Then the mixture was cooled down to room temperature. The solvent was removed under reduced pressure and the residue was purified with chromatography (hexane/ethyl acetate = 5:1-1:1) to yield a red oil intermediate, which was dissolved in toluene (5 mL). The resulting mixture was reflexed overnight. Then the mixture was cooled down to room temperature. The solvent was reduced pressure and the residue was purified with chromatography (hexane/ethyl acetate = 5:1-1:1) to yield a red oil intermediate, which was dissolved in toluene (5 mL). The resulting mixture was reflexed overnight. Then the mixture was cooled down to room temperature. The solvent was removed under reduced pressure and the residue was purified with chromatography (hexane/ethyl acetate = 5:1-2:1) to yield the product as a red soild (15 mg, 11.4%). <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  7.64 (d, *J* = 8.5 Hz, 2H), 7.54 (s, 1H), 7.08-7.16 (m, 5H), 7.01 (d, *J* = 7.5 Hz, 2H), 6.79-6.88 (m, 6H), 6.54 (d, *J* = 8.5 Hz, 2H), 6.49 (d, *J* = 8.5 Hz, 2H), 3.45 (t, *J* = 7.0 Hz, 2H), 3.41 (t, *J* = 7.0 Hz, 2H), 3.02 (s, 3H), 2.86 (s, 6H), 2.85 (s, 6H), 1.83-1.89 (m, 2H); HRMS (ESI), calcd for [M+H]<sup>+</sup>: 664.3288, found: 664.3281.

#### 9 Synthesis of TPECA-ER



To the solution of 11 (3.0 mg, 4.5 µmol) in DMSO (0.4 mL) was added ER peptide

(5.0 mg, 5.1 µmol), DIEA (0.65 mg, 5.0 µmol) and TPP (1.5 mg, 5.7 µmol). The resulting mixture was stirred at room temperature for 4 hours. Then mixture was purified with HPLC with using reverse column to give the product as red solid (1.8 mg, 25.0%). <sup>1</sup>H NMR (400 MHz, DMSO- $d_6$ )  $\delta$  8.06-8.27 (m, 6H), 7.90 (m, 1H), 7.79 (m, 1H), 7.52-7.67 (m, 5H), 7.05-7.24 (m, 16H), 6.93-6.99 (m, 2H), 6.73-6.81 (m, 4H), 6.46-6.53 (m, 4H), 4.42-4.57 (m, 5H), 4.26-4.31 (m, 3H), 4.15-4.21 (m, 2H), 3.38 (m, 3H), 2.94-3.05 (m, 5H), 2.67-2.90 (m, 20H), 2.53-2.55 (m, 1H), 2.22-2.27 (m, 2H), 1.91 (m, 1H), 1.75-1.81 (m, 5H), 1.49-1.68 (m, 8H), 1.31 (m, 2H), 0.89 (d, *J* = 6.8 Hz, 3H), 0.83 (d, *J* = 6.8 Hz, 3H). HRMS (ESI), calcd for [M+2H]<sup>2+</sup>/2: 803.8761, found: 803.8742.

#### 10. Collection of UV absorption spectra and PL spectra

UV absorption spectra were recorded in the UV-visible spectrophotometer (SHIMADZU, UV-1700). PL spectra were recorded in the luminescence spectrometer (PerkinElmar, LS 55). All the measurements were carried out at room temperature.

#### 11. Cell culture

HeLa cells were provided by American Type Culture Collection (ATCC). The cells were cultured in DMEM (Invitrogen, Carlsbad, CA) containing 10% heat-inactivated FBS (Invitrogen), 100 U mL<sup>-1</sup> penicillin, and 100  $\mu$ g mL<sup>-1</sup> streptomycin (Thermo Scientific) and were maintained in a humidified incubator at 37 °C with 5% CO<sub>2</sub>. Before experiments, the cells were pre-cultured until confluence was reached.

#### 12. Intracellular localization of TPECA-ER

HeLa cells were sub-cultured in the 8-well chambers (LAB-TEK, Chambered Coverglass System) at a density of  $5 \times 10^5$  per mL for 18 h. The culture medium was removed, and the cells were rinsed with 1×PBS. The cells were incubated with **TPECA-ER** (5 µM) for 30 min, followed by incubation with ER tracker (500 nM) for 30 min at 37 °C. After washing with 1×PBS for twice, the cells were imaged by confocal laser scanning microscope (Leica SP8, Germany). The excitation wavelength for ER tracker and **TPECA-ER** were 405 nm and 488 nm, respectively. The fluorescence signal were collected at the range of 420-500 nm (ER tracker) and 550-

#### 800 nm (TPECA-ER).

#### 13 Cell cytotoxicity assay

The cytotoxicity of **TPECA-ER** in dark or under light irradiation was evaluated using the MTT assay. The HeLa cells were seeded in 96-well plates at a density of  $1 \times 10^5$ cells/well. After incubation for 24 h, the medium was replaced with freshly prepared medium containing different concentrations of **TPECA-ER**. The cells were further incubated at 37 °C for 2 h in the dark and washed with 1× PBS for three times. After adding fresh medium, the cells were irradiated under white light for 3 min at the power of 0.25 W cm<sup>-2</sup> while the control groups were kept under dark environment. The cells were further incubated for 24 h and washed with 1×PBS. 100 µL of freshly prepared MTT (0.5 mg mL<sup>-1</sup>) solution in 1×PBS was added into each well and kept at 37 °C for 3 h. The MTT medium was removed and the formazan crystals were dissolved in DMSO. The cell viability was calculated by measuring the absorbance at 570 nm using a microplate reader (Genios Tecan).

#### 14 Photostability test

HeLa cells were cultured in the chambers (LAB-TEK, Chambered Coverglass System) at a density of  $5 \times 10^5$  per mL for 18 h. The culture medium was removed, and the cells were rinsed with 1×PBS. Two groups of cells were incubated with **TPECA-ER** (5  $\mu$ M) or ER tracker (500 nM) for 30 min at 37 °C, respectively. After washing with 1×PBS for 3 times, the cells were fixed by 4% formaldehyde and scanned by confocal laser scanning microscope (Leica SP8, Germany) for 60 times. The excitation wavelength for ER tracker and **TPECA-ER** were 405 nm and 488 nm, respectively. The fluorescence signal were collected at the range of 420-500 nm (ER tracker) and 560-800 nm (**TPECA-ER**). The change of fluorescence intensity after each scan were measured and plotted to reveal the photostability of ER tracker and **TPECA-ER**.



**Figure S1** A) UV-vis absorption spectra of NTPE-DCV in water (10  $\mu$ M). B) The absorption spectra of ABDA (150  $\mu$ M) in the presence of NTPE-DCV (10  $\mu$ M) after different durations of white light irradiation. C) UV-vis absorption value at 400 nm for ABDA (150  $\mu$ M) at different irradiation time.



Figure S2 Mass spectrum of reaction mixture between NTPE-DCV and homo-Cysteine.



Figure S3 Mass spectrum of reaction mixture between NTPE-DCV and Cysteine.



Figure S4 Mass spectrum of reaction mixture between NTPE-DCV and GSH.



Figure S5 <sup>1</sup>H-NMR of 2-cyano-N,N-diethylacetamide in CDCl<sub>3</sub>







Figure S8  $^{\rm 13}\text{C-NMR}$  of compound 3 in  $CD_3CN$ 



Figure S9 High resolution mass spectrum of compound 3



Chemical shift (ppm) Figure S10 <sup>1</sup>H-NMR of compound 3 in DMSO- $d_6$ 



Figure S11 High resolution mass spectrum of compound 4

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Chemical shift (ppm) Figure S12 <sup>1</sup>H-NMR of compound 5 in CD<sub>3</sub>CN



Figure S13 High resolution mass spectrum of compound 5



Figure S14 <sup>1</sup>H-NMR of compound 6 in DMSO-*d*<sub>6</sub>



Figure S15 High resolution mass spectrum of compound 6



Figure S16 HMBC spectrum of fluorophore 5.



**Figure S17** Calculated HOMO and LUMO energy state for fluorophores NTPE-DCV, 3, 4, 5, and 6.



**Figure S18** Photoluminescence (PL) spectra of 6 (12.5  $\mu$ M) in DMSO–water mixtures with different fractions of water ( $f_w$ ).



**Figure S19** Value of absorption for fluorophores (NTPE-DCV, 3-6) incubating with six-fold cysteine. The absorption wavelength used in the calculation of  $A/A_0$  for is 510 nm, 484 nm, 485 nm, 442 nm, 426 nm respectively.



**Figure S20** ΔEst value calculated by DFT for fluorophores NTPE-DCV, 3, 4, 5 and 6.









Figure S25 <sup>1</sup>H-NMR of compound 10 in CDCl<sub>3</sub>



Figure S26 High resolution mass spectrum of compound 10

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Figure S27 <sup>1</sup>H-NMR of compound 11 in CDCl<sub>3</sub>



Figure S28 High resolution mass spectrum of compound 11



Figure S29 UV-vis absorption spectra of 11 (20  $\mu$ M) in PBS buffer (1 $\times$ ) at different pH value.





Chemical shift (ppm)

Figure S30 <sup>1</sup>H-NMR of compound TPECA-ER in DMSO-*d*<sub>6</sub>



Figure S31 High resolution mass spectrum of compound TPECA-ER



**Figure S32** UV-vis absorption spectra of 5 and TPECA-ER in DMSO/PBS (v/v = 1/99).



Figure S33 Viability of HeLa cells upon treatment with TPECA-ER at various concentration in dark or under different duration of white light irradiation (0.25 W  $cm^{-2}$ , 3 min).



**Figure S34** Photostability of TPECA-ER and commercial ER tracker. I0 is the initial fluoroscent intensity of TPECA-ER and commercial ER tracker. I is the fluoroscent intensity of TPECA-ER and commercial ER tracker after a certain number of scans.



**Figure S35** Co-localization scatter plots for ER tracker (Fig 3D) and TPECA-ER (Fig 3E).