Supporting Information

An Easily Available Ratiometric AIE Probe for Nitroxyl Visualization *in Vitro* and *in Vivo*

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General procedures for the detection of HNO

Unless otherwise noted, all the spectral measurements were performed in 5 mM phosphate buffer (pH = 7.4) according to the following procedure. The stock solution (1.0 mM) of probe TCFPB-HNO was first prepared in DMSO. 10 μL of TCFPB-HNO stock solution was added to 2 mL PBS followed by addition of different volume of AS solution. The mixture was incubated for different time at 37 °C and then the reaction solutions were transferred to a quartz cell with 1 cm optical length for measurements. In the meantime, the blank solution without HNO was prepared and measured under the same conditions for comparison. HNO was prepared from Angeli's salt in aqueous solution. Hydrogen peroxide (H_2O_2) and hypochlorite (ClO^-) were delivered from 30% and 5% aqueous solutions, respectively. Hydroxyl radical (•OH) was generated by reaction of 100 μM Fe^{2+} with 100 μM H_2O_2.

Calculation of the detection limit of TCFPB-HNO toward addition of HNO

Based on the linear fitting in Fig. 2C, the detection limit (C) is estimated as follows:

$$C = 3\sigma/B$$

Where σ is the standard deviation obtained from three individual fluorescent intensity ratio (I_{618}/I_{710}) of TCFPB-HNO (5 μM) without any HNO and B is the slope obtained after linear fitting the titration curves within certain ranges.

Cell cultures

The MCF-7 cells were cultured in DMEM (containing 10% heat-inactivated FBS, 100 mg·mL^{-1} penicillin and 100 mg·mL^{-1} streptomycin) at 37 °C in a humidified incubator with 5% CO_2. Before the experiments, the cells were precultured until confluence was reached.

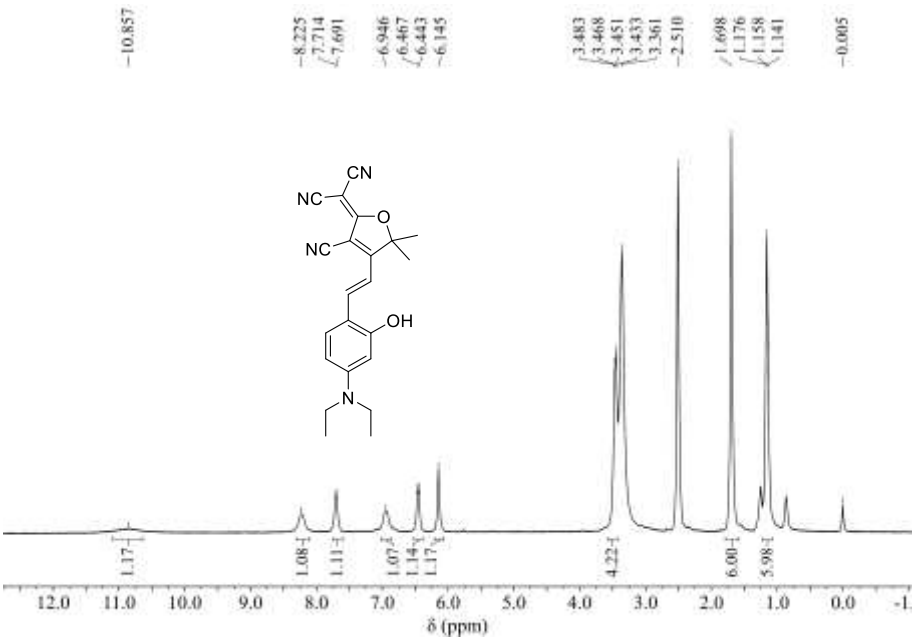

Fig. S1. ^{1}H NMR spectrum of TCFIS in DMSO-d_6.

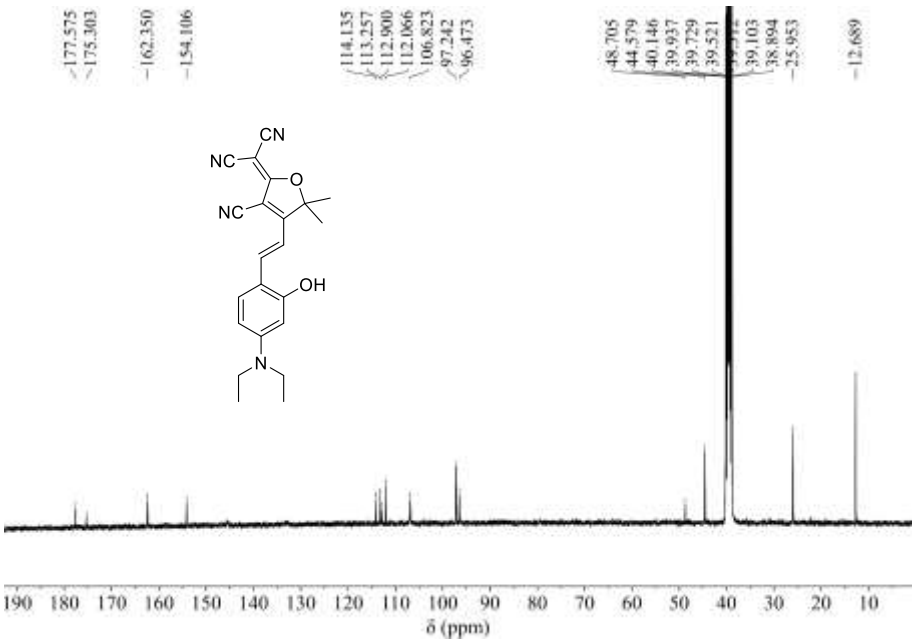

Fig. S2. ^{13}C NMR spectrum of TCFIS in DMSO-d_6.

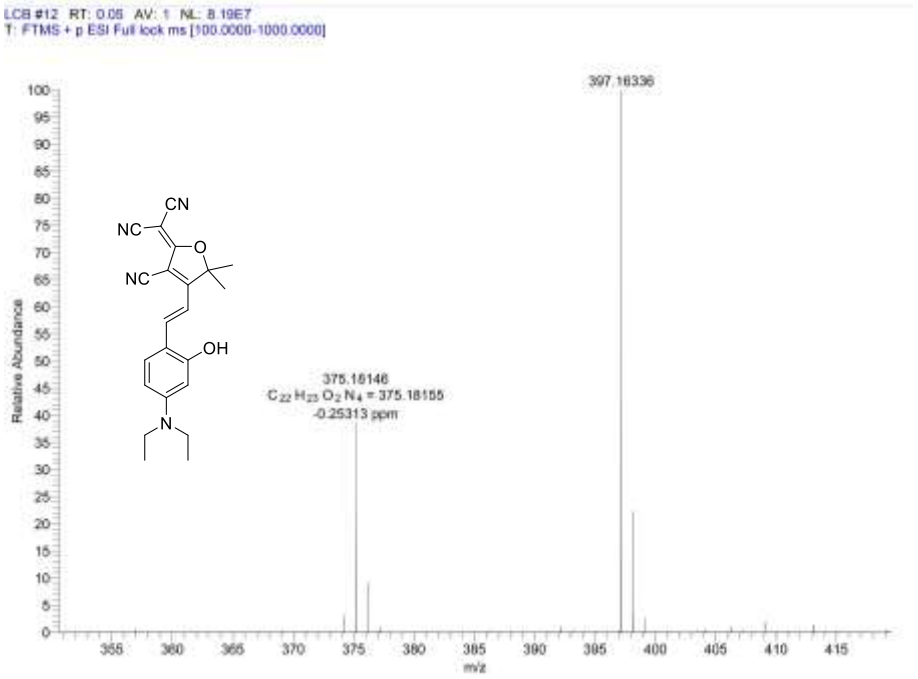

Fig. S3. HR-MS spectrum of TCFIS.

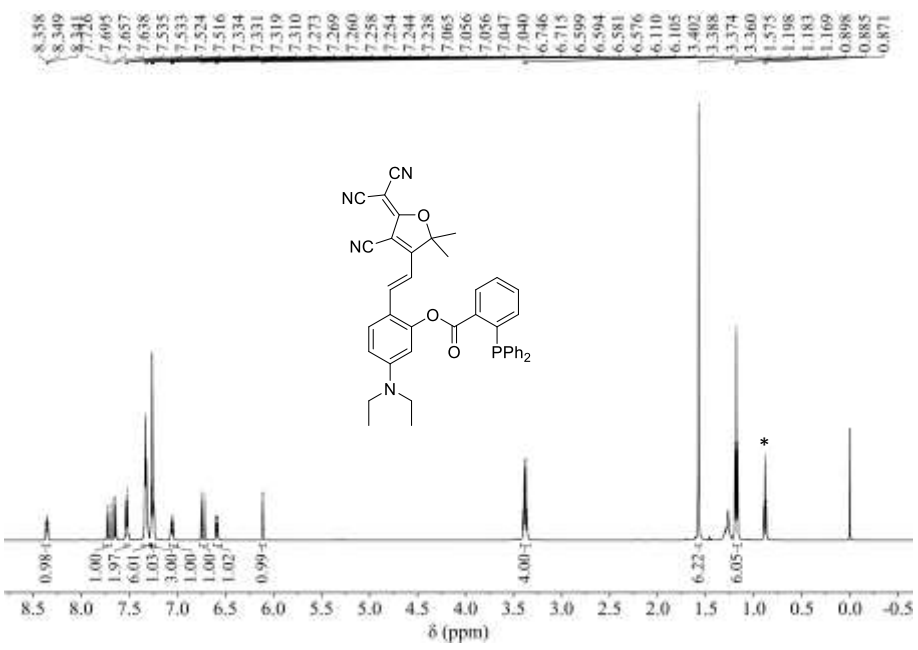

Fig. S4. ^{1}H NMR spectrum of TCFPB-HNO in $CDCl_3$.

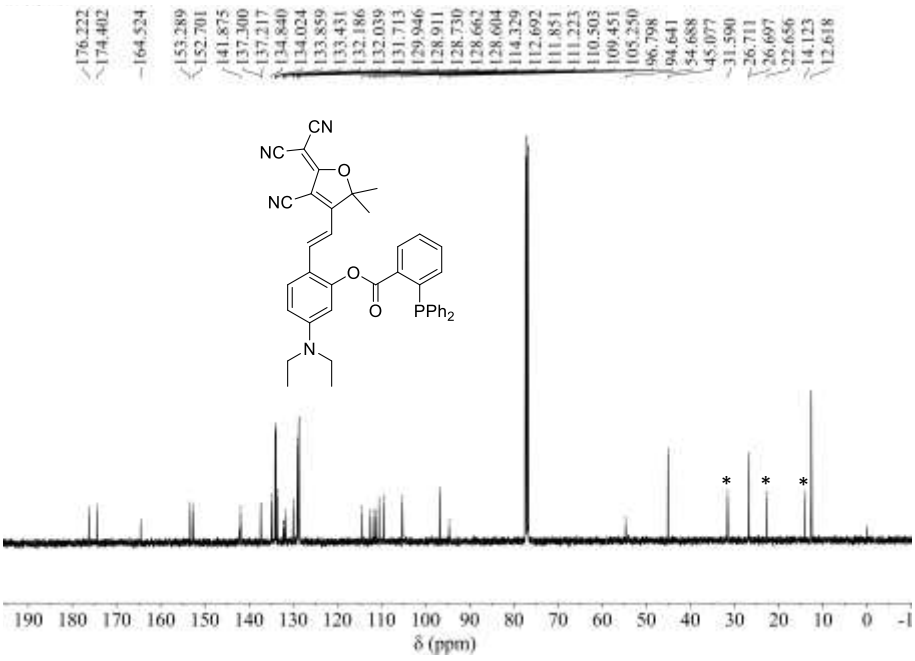

Fig. S5. ^{13}C NMR spectrum of TCFPB-HNO in $CDCl_3$.

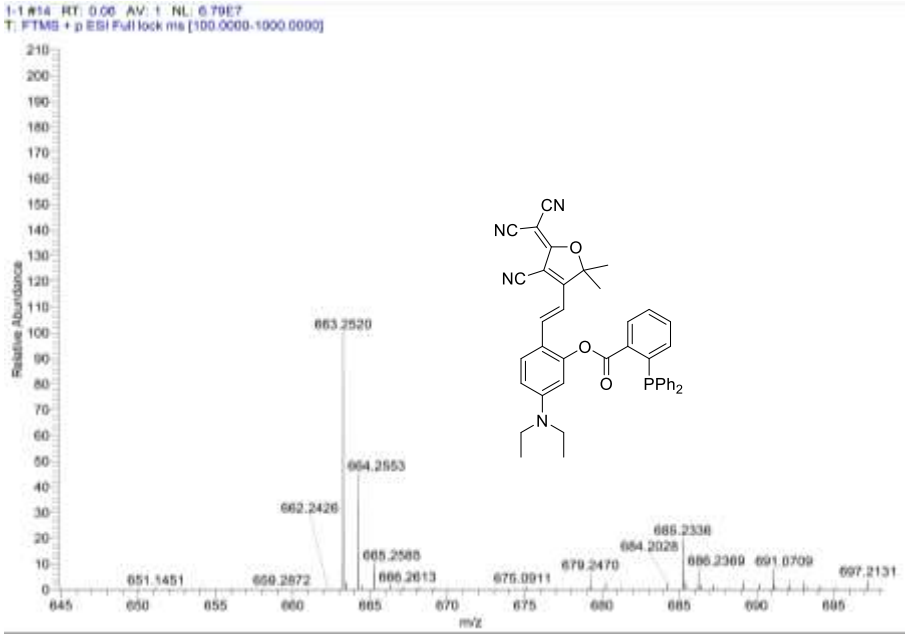

Fig. S6. HR-MS spectrum of TCFPB-HNO.

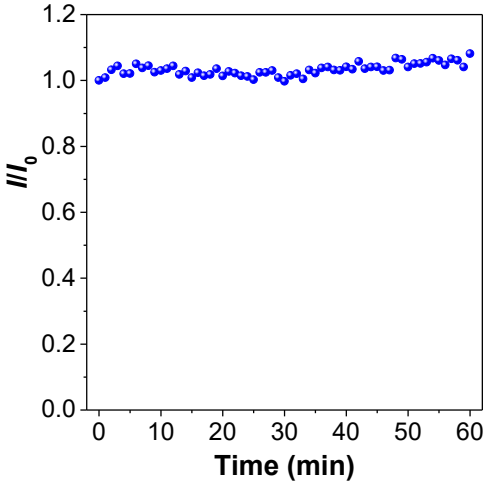

Fig. S7. Photostability of TCFPB-HNO in PBS solution.

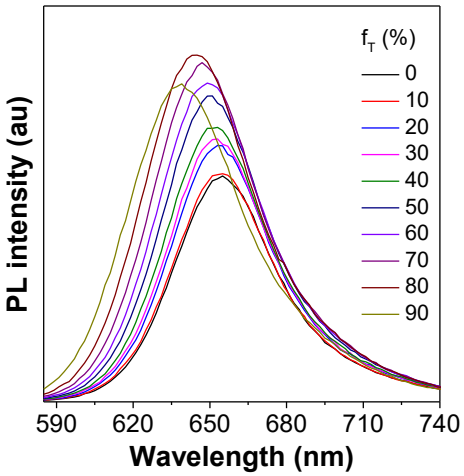

Fig. S8. PL spectra of TCFPB-HNO in toluene/DMSO mixtures with different toluene fractions (f_T).

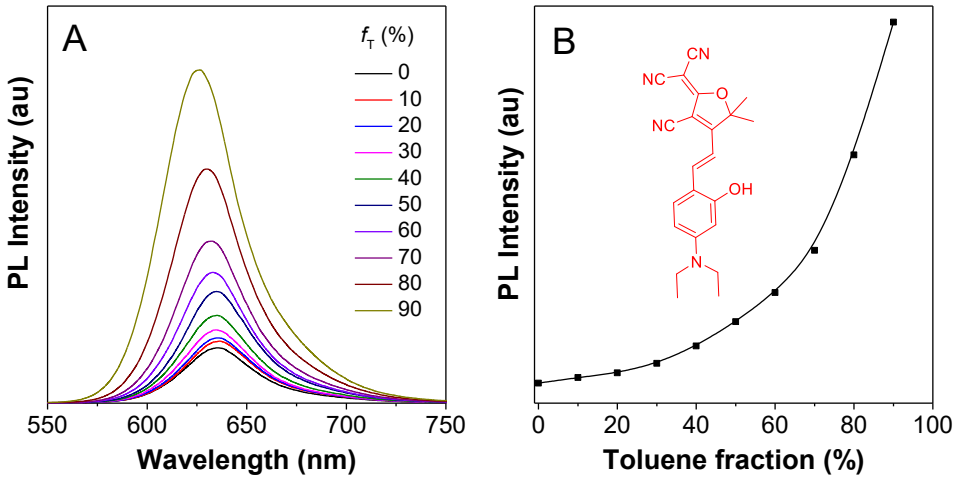

Fig. S9. (A) PL spectra of TCFIS in toluene/DMSO mixtures with different f_T. (B) Plot of PL intensity of TCFIS at maximum emission wavelength *vs.* f_T in the toluene/DMSO mixtures. E_x = 540 nm.

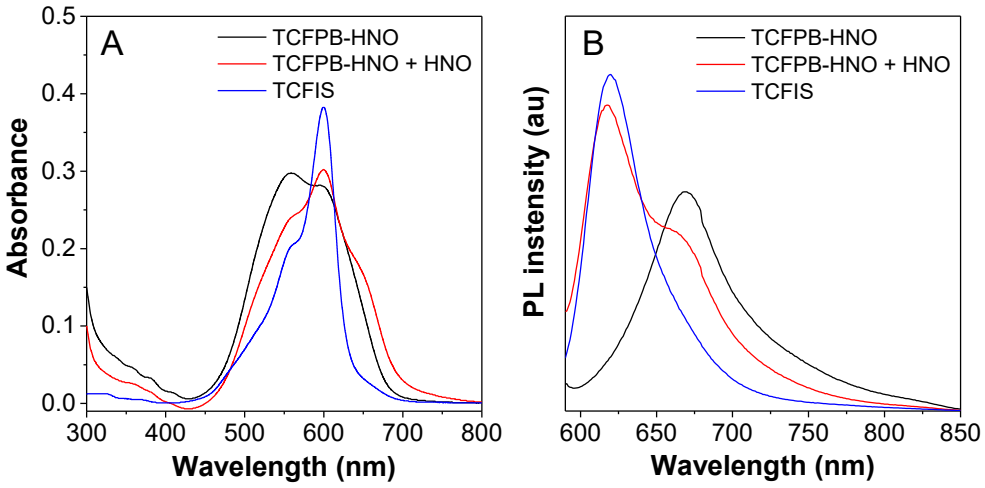

Fig. S10. (A) UV-*vis* spectra and (B) PL spectra of TCFPB-HNO (5 μM) and TCFPB-HNO + AS (50 μM) at 37 ℃ for 40 min in PBS solution. Excitation wavelength (E_x) = 570 nm.

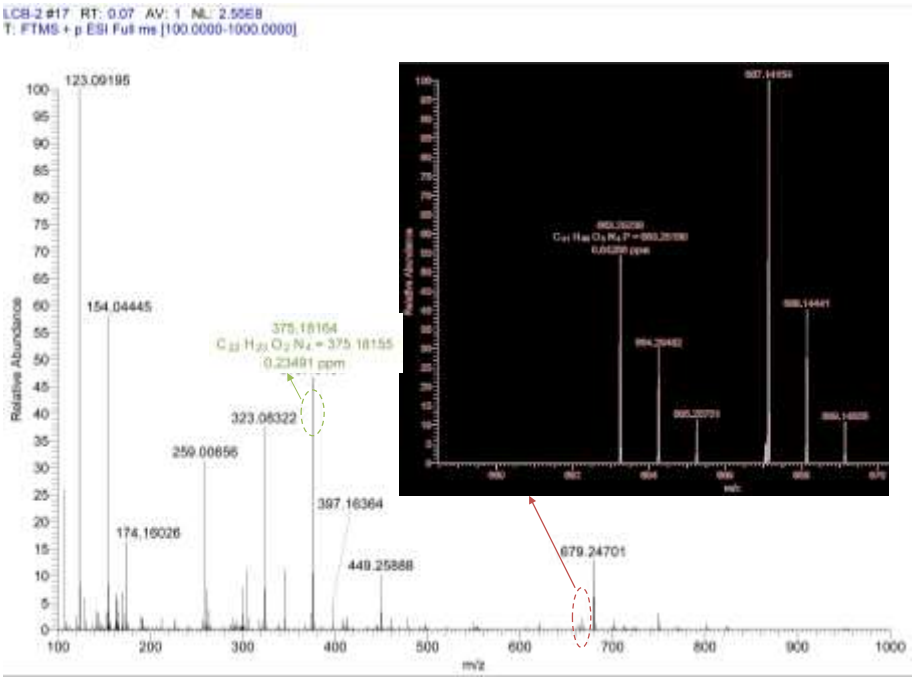

Fig. S11. HRMS spectrum of TCFPB-HNO after incubation with AS (150 μM) at 37 ℃ for 40 min.

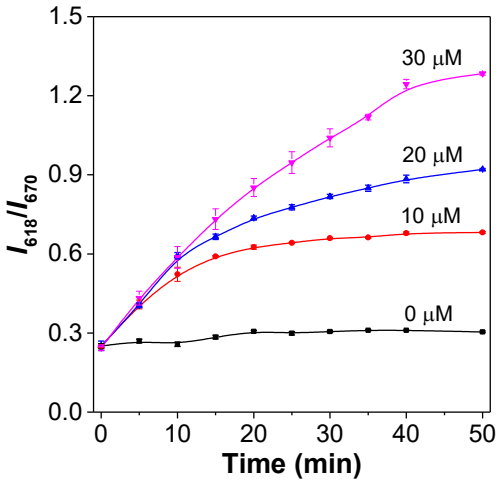

Fig. S12. PL intensity ratio (I_{618}/I_{670}) of TCFPB-HNO (5 μM) in the presence of different concentrations of AS as a function of incubation time. E_x = 570 nm.

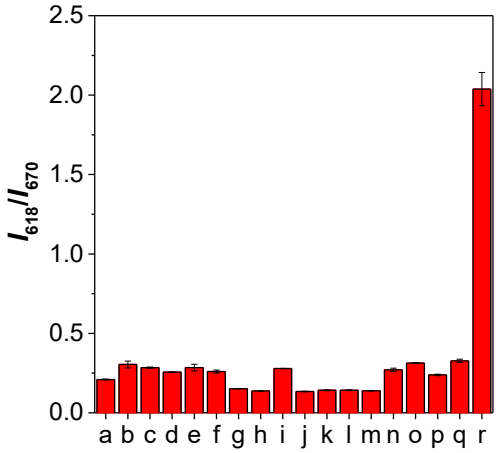

Fig. S13.Variations of PL intensity ratio (I_{618}/I_{670}) of TCFPB-HNO (5 μM) after incubation with 50 μM of AS or 100 μM of other biologically-relevant species. a: blank; b: ClO^-; c: H_2O_2; d: TBHP; e: •OH; f: $•O_2^-$; g: Hcy; h: GSH; i: Cys; j: Fe^{3+}; k: Na^+; l: K^+; m: Mg^{2+}; n: Zn^{2+}; o: NO_2^-; p: NO_3^-; q: S^{2-}; r: HNO. E_x = 570 nm.

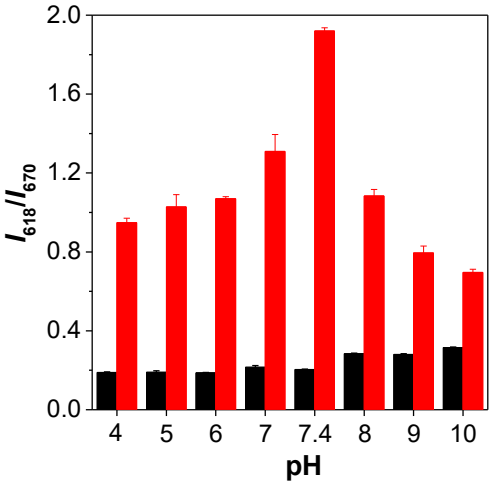

Fig. S14.The PL intensity ratio (I_{618}/I_{670}) of TCFPB-HNO (5 μM, black bars) and TCFPB-HNO (5 μM) + AS (50 μM, red bars) in different pH buffers. E_x = 570 nm.

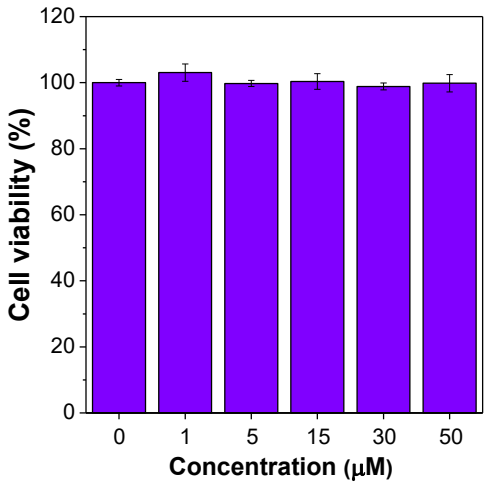

Fig. S15. Cell viability of MCF-7 cells at varied concentrations of TCFPB-HNO by CCK-8 assay.

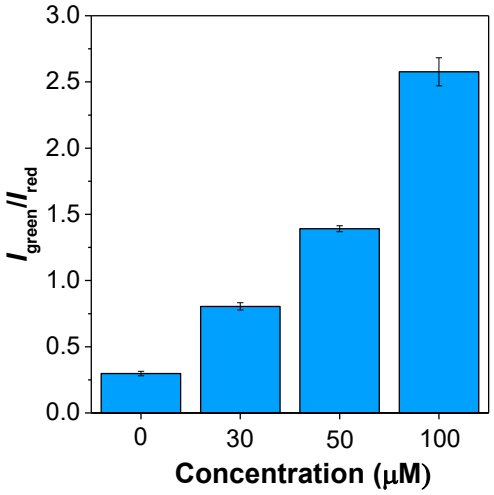

Fig. S16. The PL intensity ratio (I_{green}/I_{red}) of the green channel and red channel in Fig. 3.

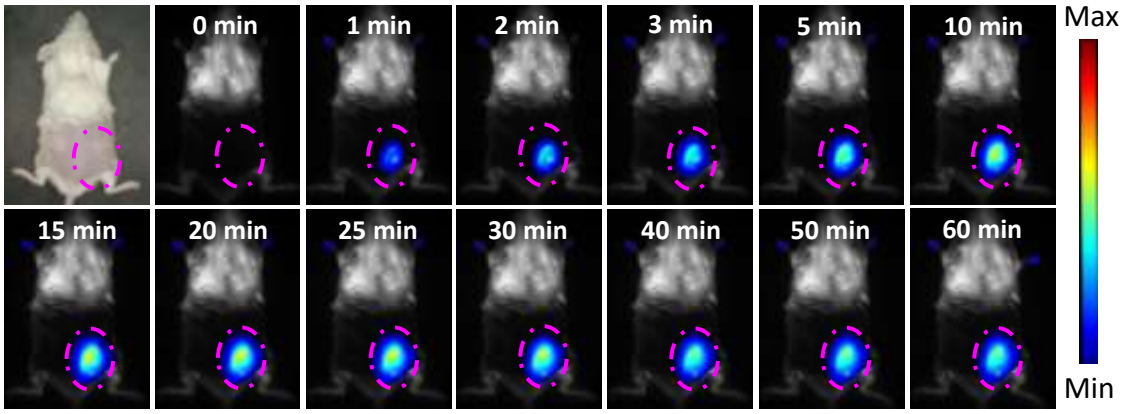

Fig. S17. Time-dependent fluorescent images in live mice using probe TCFPB-HNO in the absence of HNO.

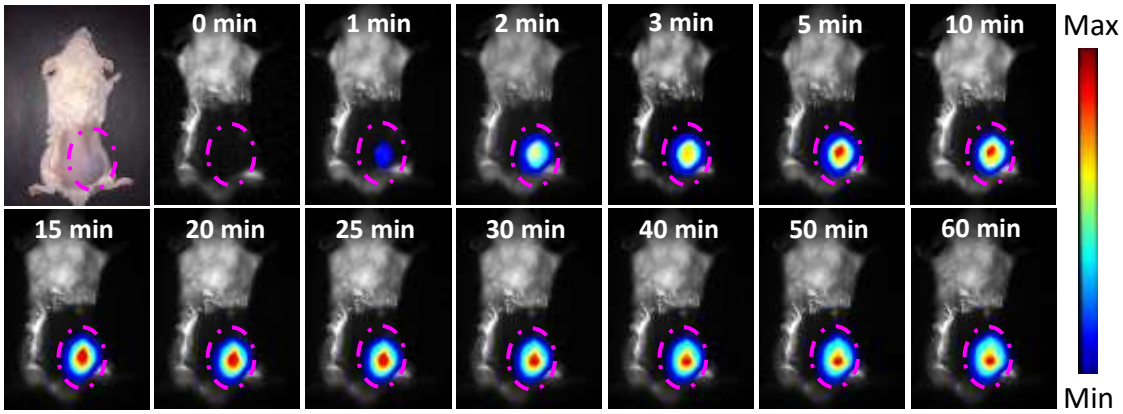

Fig. S18. Time-dependent fluorescent images of HNO in live mice using probe TCFPB-HNO in the presence of HNO.

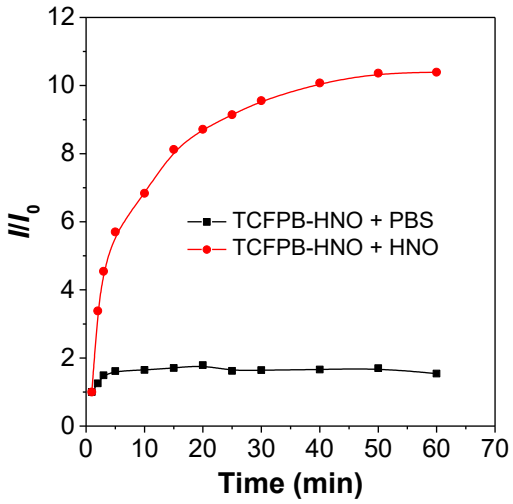

Fig. S19. Time-dependent changes of average PL intensity ratio (I/I_0) in Fig. S17 and Fig. S18, where I_0 is the average PL intensity of TCFPB-HNO at 0 min in Fig. S17 and Fig. S18 respectively; and I is the average PL intensity of TCFPB at t min in Fig. S17 and Fig. S18 respectively.

Table S1. Comparison of some fluorescent probes for HNO detection.

References	Response mode	Response time	Detection limit	λ_{em} (nm)	Imaging application
Anal. Chem., 89 (**2017**) 12087	Turn-on (ACQ)	ND	2000 nM	585	cell
Chem. Commun., 50 (**2014**) 5790	Turn-on (ACQ)	30 min	20 nM	450	Serum
Chem. Commun., 50 (**2014**) 14253	Turn-on (ACQ)	20 min	60 nM	700	cell
Chem. Commun., 51 (**2015**) 5754	Turn-on (ACQ)	45 min	59 nM	512	cell
Analyst, 140 (**2015**) 4576	Turn-on (ACQ)	20 min	30 nM	734	cell/mice
Sensors Actuat. B-Chem., 220 (**2015**) 727	Turn-on (ACQ)	5 min	50 nM	459	cell
RSC Adv., 5(**2015**) 84543	Turn-on (ACQ)	30 min	20 nM	590	cell/nematode
J. Mater. Chem. B, 4 (**2016**) 1263	Turn-on (ACQ)	40-45 min	608-648 nM	**1.** 490 **2.** 635 **3.** 710	cell
J. Mater. Chem. B, 5 (**2017**) 1954	Turn-on (ACQ)	45 min	170 nM	545	cell
J. Mater. Chem. B, 5 (**2017**) 5218	Turn-on (ACQ)	20 min	65 nM	415	cell/tissue
Bioorg. Med. Chem. Letters, 25 (**2015**)16	Turn-on (ACQ)	40 min	ND	520	cell
Dyes and Pigments, 131 (**2016**) 24	Turn-on (ACQ)	12 min	60 nM	727	cell
Sensors Actuat. B-Chem., 224 (**2016**) 209	Turn-on (ACQ)	50 min	128 nM	520	serum
J. Am. Chem. Soc., 135 (**2013**) 12690	Turn-on (ACQ)	30 min	ND	526	cell
Chin J Anal Chem, 43 (**2015**) 1829	Turn-on (ACQ)	40 min	30 nM	710	cell
Sci. Rep., 5 (**2015**) 16979	Turn-on (ACQ)	30min	43 nM	696	cell
Anal. Chem. 90 (**2018**) 4641	Turn-on (ACQ)	20 min	190 nM	550	cell
Dyes and Pigments, 148 (**2018**) 348	Turn-on (ACQ)	ND	ND	510	cell
J. Mater. Chem. B, 5 (**2017**) 3557	Turn-on (ACQ)	2 min	10 nM	614	cell

Chem. Commun., 53 (**2017**) 1723	Turn-on (ACQ)	2 min	10 nM	542	cell
Talanta Volume, 193 (**2019**) 152	Turn-on (ACQ)	10 min	50 nM	542	cell
Sensors Actuat. B-Chem., 312 (**2020**) 127944	Turn-on (ACQ)	5 min	116.3 nm	520, 673	cell
Spectrochim. Acta Mol Biomol. Spectros., 241 (**2020**) 118680	Turn-on (ACQ	15 min	12 nM	740	cell
Anal. Methods, 11(**2019**) 832	Turn-on (ACQ	12 min	43 nM	500	cell
Dalton Trans., 48 (**2019**) 2760	Turn-on (ACQ	ND	410 nM	543	cell
Anal. Chem. 91 (**2019**) 4451	Turn-on (ACQ)	3 min	160 nM	610	cell
Sensors Actuat. B-Chem., 310 (**2020**) 127839	Turn-on (ACQ)	400 s	15 nM	775	cell
Sensors Actuat. B-Chem., 317 (**2020**) 128211	Turn-on (ACQ)	2 min	32 nM	555	cell
Sci. China Chem., 63 (**2019**) 282	Turn-on (ACQ)	4 min	13 nM	700	cell/tissue / live mice imaging
Spectrochim. Acta Mol Biomol. Spectros., 227 (**2020**) 117765	Turn-on (ACQ)	10 min	39.6 nM	688	cell
Chem. Commun., 55 (**2019**) 8583	Turn-on (ACQ)	5 min	90 nM	676	cell
Dyes and Pigments 185 (**2020**) 108889	Turn-on (ACQ)	10 min	9 nM	520	cell
Talanta 206 (**2020**) 120196	Turn-on (ACQ)	20 min	1000 nM	670	cell
Anal. Methods, 11 (**2019**) 1299	Turn-on (ACQ)	20 min	78 nM	432	cell
ACS Appl. Mater. Interfaces, 7 (**2015**), 5438	Ratiometric (ACQ)	15 min	1400 nM	517/470	cell
Chem. Commun., 50 (**2014**) 6013	Ratiometric (ACQ)	20 min	500 nM	546/418	cell
Anal. Chem., 89 (**2017**) 4587	Ratiometric (ACQ)	15 min	50 nM	540/470	cell
Sensors Actuat. B-Chem., 233 (**2016**) 193	Ratiometric (ACQ)	45 min	27 nM	592/463	cell
Anal. Chem., 290 (**2018**) 3914	Ratiometric (ACQ)	80 s	8.82 nM	568/601	cell

Anal. Methods, 7 (**2015**) 3883	Ratiometric (ACQ)	15 min	980 nM	460/380	cell
Chem. Commun., 52 (**2016**) 733	Ratiometric (ACQ)	25 min	590 nM	541/448	cell/tissue
This work	**Ratiometric (AIE)**	**40 min**	**157.6 nM**	**618/670**	**cell/mice**