

## Supporting Information

# **Azo-Based Hypoxia Responsive Self-Assembly Near-Infrared Fluorescent Nanoprobe for in Vivo Real-Time Bioimaging of Tumor**

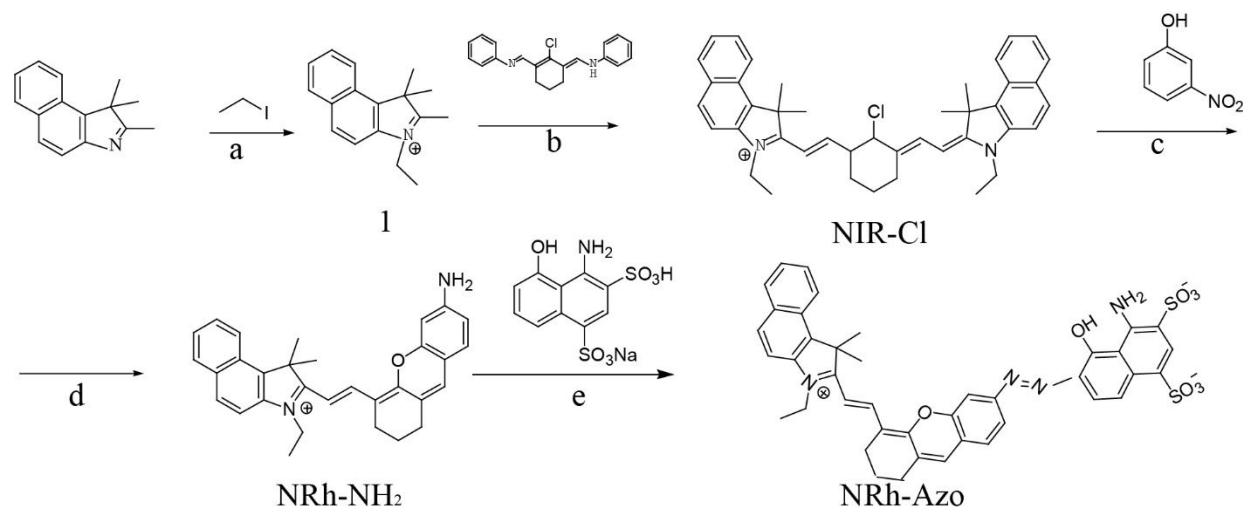
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**Scheme S1.** Synthesis of **NRh-Azo**. Conditions: a) toluene reflux, 130 ° C, 8 h; b) acetic anhydride, potassium acetate, 60 °C, 3 h; c) DMF, trimethylamine, 85 °C, 3 h; d) SnCl<sub>2</sub>, HCl, 85 °C, 8 h; e) HCl, NaNO<sub>2</sub>, NaHCO<sub>3</sub>, 0 °C, 1 h.

**compound 1.** 1,1,2-Trimethylbenz[e]indole (2.09 g 10.0 mmol) and iodoethane (1.56 g 10.0 mmol) were placed in a flask containing dry toluene (15.0 ml), and the mixture was stirred at 130°C with solvent reflux under a nitrogen atmosphere for 8 hours. The solution was removed under vacuum filtration and washed with ether to produce compound 1 as a blue solid (2.92 g, yield 80%). <sup>1</sup>H NMR (400MHz, MeOD) δ 8.34 (t, *J* = 12.6 Hz, 1H), 8.27 (d, *J* = 8.9 Hz, 1H), 8.19 (d, *J* = 8.2 Hz, 1H), 8.05(d, *J* = 8.9 Hz, 1H), 7.83 (t, *J* = 7.6 Hz, 1H), 7.74 (t, *J* = 7.5 Hz, 1H), 4.89 (s, 3H), 4.70 (q, *J* = 7.3Hz, 2H), 1.87 (s, 6H), 1.66 (t, *J* = 7.3 Hz, 3H). MS (ESI<sup>+</sup>): calcd for C<sub>17</sub> H<sub>20</sub>N<sup>+</sup>, 238.2 [M]<sup>+</sup>; found, 238.1589 [M]<sup>+</sup>.

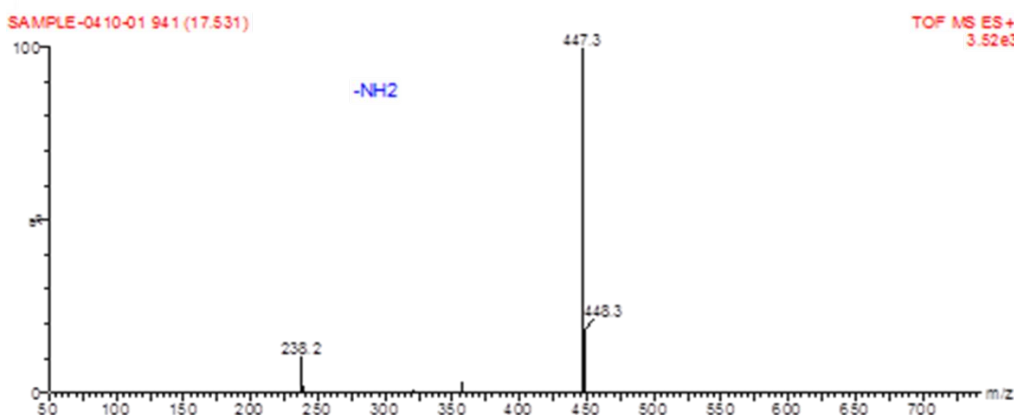
**NIR-Cl.** Compound 1 (2.92 g, 8.0 mmol) and N-((2-Chloro-3((phenylimino) methyl) cyclohex-2en-1-ylidene) methyl) aniline hydrochloride (1.44 g, 4.0 mmol) was added into a flask containing dryacetic anhydride and potassium acetate under a nitrogen atmosphere. The mixture was stirred at 60°C for 3 hours. The solution was hydrolysed by sodium bicarbonate and removed under vacuum filtration to produce NIR-Cl as a red solid (2.35 g, yield 80%). <sup>1</sup>H NMR

(400 MHz, MeOD)  $\delta$  8.90(d,  $J$  = 15.3 Hz, 1H), 8.43 (d,  $J$  = 7.9 Hz, 1H), 8.18 (d,  $J$  = 8.9 Hz, 1H), 8.11 (t,  $J$  = 7.3 Hz, 1H), 7.86 (d,  $J$  = 8.9 Hz, 1H), 7.78 (ddd,  $J$  = 8.4, 6.9, 1.2 Hz, 1H), 7.68 – 7.62 (m, 1H), 7.53 (d,  $J$  = 8.3 Hz, 1H), 7.30 (s, 1H), 7.25 – 7.19 (m, 1H), 7.13 – 7.02 (m, 1H), 6.68 (d,  $J$  = 15.2 Hz, 1H), 4.60 (q,  $J$  = 7.3 Hz, 2H), 2.80 (dt,  $J$  = 11.9, 5.9 Hz, 4H), 2.12 (d,  $J$  = 5.1 Hz, 6H), 1.99 (dd,  $J$  = 10.5, 4.4 Hz, 2H), 1.59 (d,  $J$  = 7.3 Hz, 2H). MS (ESI +): calcd for  $C_{42}H_{44}ClN_2^+$ , 611.3 [M]<sup>+</sup>; found, 611.3186 [M]<sup>+</sup>.

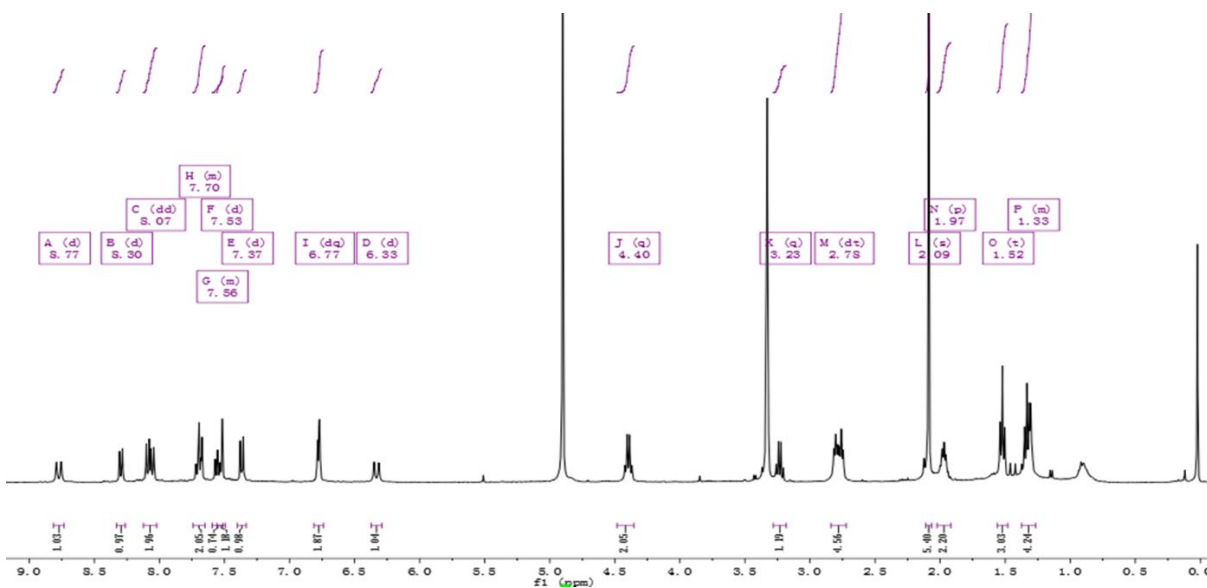
**NRh-NH<sub>2</sub>.** NIR-Cl (1.47 g, 2.0 mmol) and 3-Nitrophenol (0.42 g, 3.0 mmol) were placed in a flask containing dry N, N-dimethylformamide and triethylamine under a nitrogen atmosphere at 85°C for 3 hours. SnCl<sub>2</sub> (3.80 g, 20.0 mmol) dissolved in concentrated HCl (4.0 mL) was added to the above solution under nitrogen atmosphere. The mixture was stirred at 85°C for 8 hours. Then the solution was removed under vacuum filtration at 85°C. The crude product was purified by silica column chromatography using CH<sub>2</sub>Cl<sub>2</sub>/CH<sub>3</sub>OH (50:1) as the eluent to produce the NRh-NH<sub>2</sub> compound as a black solid (0.72 g, yield 50%). <sup>1</sup>H NMR (400 MHz, MeOD)  $\delta$  8.77 (d,  $J$  = 14.5 Hz, 1H), 8.30 (d,  $J$  = 8.6 Hz, 1H), 8.07 (dd,  $J$  = 14.1, 8.5 Hz, 2H), 7.72 – 7.66 (m, 2H), 7.55 (t,  $J$  = 8.3, 6.9 Hz, 1H), 7.52 (s, 1H), 7.37 (d,  $J$  = 9.0 Hz, 1H), 6.77 (dd,  $J$  = 3.7, 2.1 Hz, 2H), 6.33 (d,  $J$  = 14.5 Hz, 1H), 4.40 (q,  $J$  = 7.2 Hz, 2H), 3.23 (d,  $J$  = 7.3 Hz, 1H), 2.78 (dt,  $J$  = 16.8, 6.1 Hz, 5H), 2.09 (s, 6H), 1.98 (t,  $J$  = 6.2 Hz, 2H), 1.52 (t,  $J$  = 7.2 Hz, 3H), 1.33 (q, 4H).

**NRh-Azo.** First, NRh-NH<sub>2</sub> (100 mg, 0.2 mmol) was dissolved in MeCN / H<sub>2</sub>O (1:2, 3 mL) and NaNO<sub>2</sub> (42 mg, 0.6 mmol) was dissolved in ice water (0.5 mL). Under 0 °C and argon atmosphere, ice HCl (2 M, 300  $\mu$ L) was added to the solution of NRh-NH<sub>2</sub> and stir at 0 °C for 15 min. Then, previously prepared NaNO<sub>2</sub> solution was added and stirring was continued at 0 °C for 30 min to obtain diazonium salt solution. Next, the diazonium salt solution was added to the mixture solution (1 mL, ice water) of NaHCO<sub>3</sub> (67 mg, 0.8 mmol) and 1-Amino-8-naphthol-2,4-

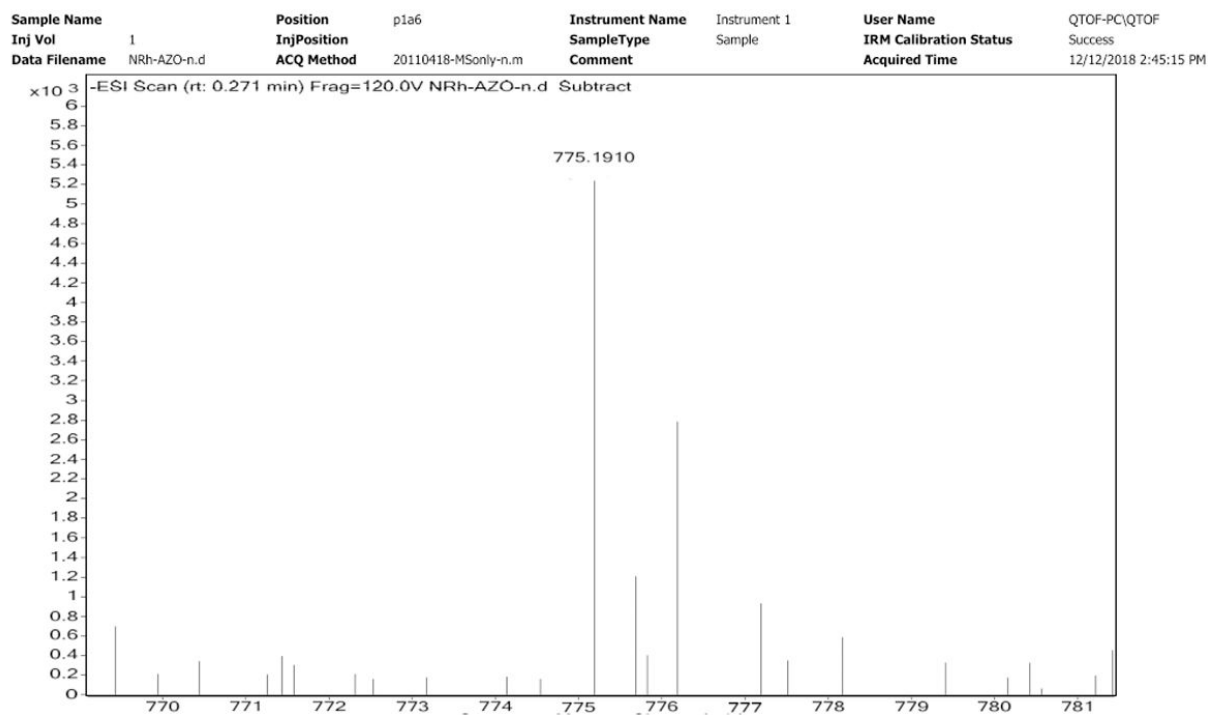
disulfonic acid monosodium salt hydrate (102mg, 0.3mmol). The resulting mixture was stirred at 0 °C for 1 h. The crude product was purified by high-performance liquid chromatography (HPLC) and lyophilized into aubergine solid (31 mg, yield 20%). The structure of NRh-Azo was further confirmed by electrospray ionization mass spectrometry (ESI-MS), and <sup>1</sup>H NMR (Figures S1–2). <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ 8.70 (d, *J* = 15.1 Hz, 1H), 8.43 – 8.37 (m, 2H), 8.23 (d, *J* = 8.9 Hz, 1H), 8.17 (d, *J* = 8.1 Hz, 1H), 8.09 (d, *J* = 9.9 Hz, 1H), 7.98 (d, *J* = 9.0 Hz, 1H), 7.77 (t, *J* = 7.7 Hz, 1H), 7.68 (s, 1H), 7.65 (d, *J* = 6.3, 3.4 Hz, 1H), 7.61 (s, 2H), 7.44 (s, 1H), 6.95 (d, *J* = 10.0 Hz, 1H), 6.63 (d, *J* = 15.3 Hz, 1H), 4.59 (d, *J* = 7.7 Hz, 2H), 2.72 (d, 4H), 2.08 (s, 6H), 1.99 (q, 2H), 0.85 (t, *J* = 7.0 Hz, 3H). MS (ESI<sup>−</sup>): *m/z* = 775.19 [*M*]<sup>−</sup>



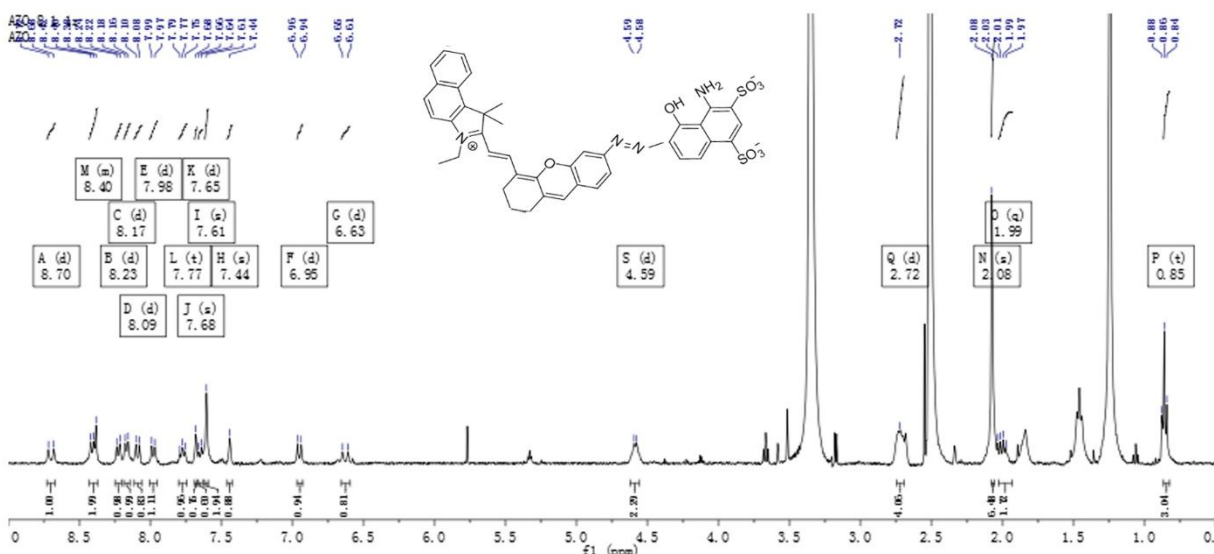
**Figure S1.** Mass spectra of NRh-NH<sub>2</sub>



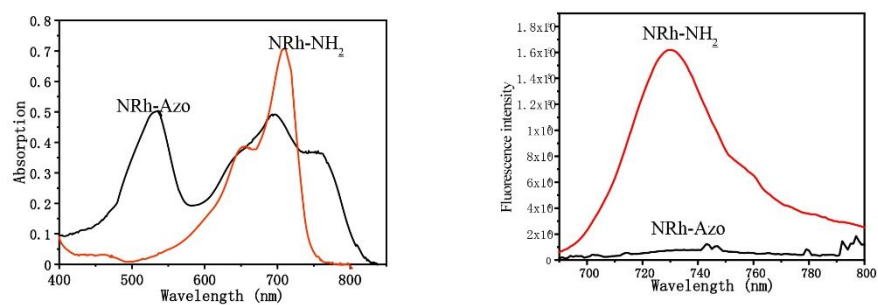
**Figure S2.** <sup>1</sup>H NMR spectra of NRh-NH<sub>2</sub> in MeOD-d<sub>4</sub>



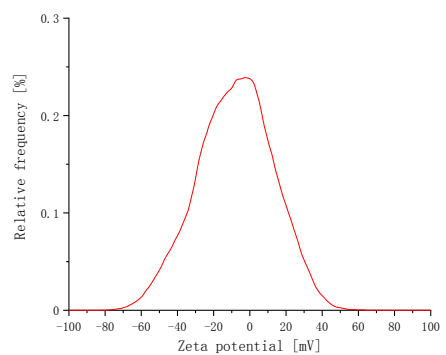
**Figure S3.** Mass spectra of NRh-Azo



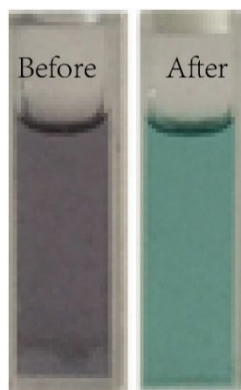
**Figure S4.**  $^1\text{H}$  NMR spectra of NRh-Azo in DMSO- $d_6$



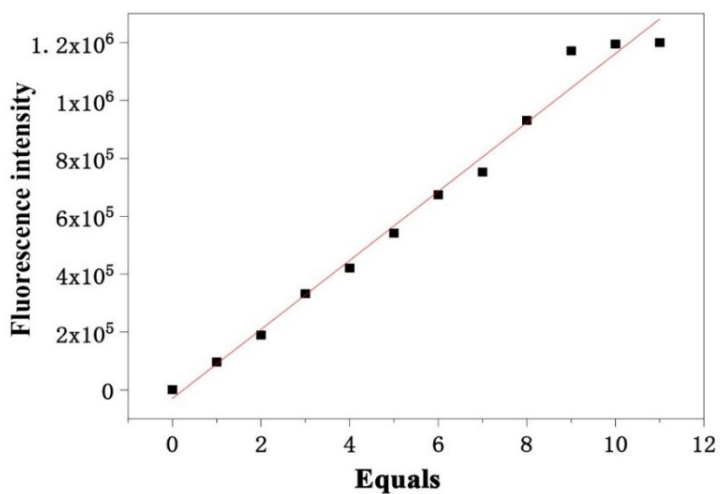
**Figure S5.** Absorption and fluorescence emission spectra of NRh-NH<sub>2</sub> and NRh-azo.



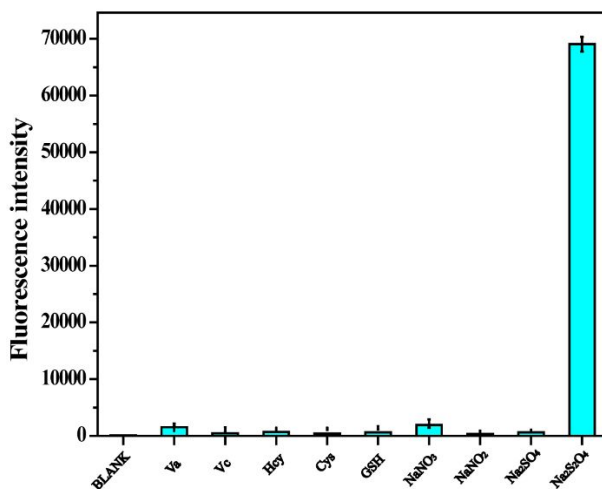
**Figure S6.** Zeta potential of NRh-azo, displaying a value of  $-11 \pm 2$  mV



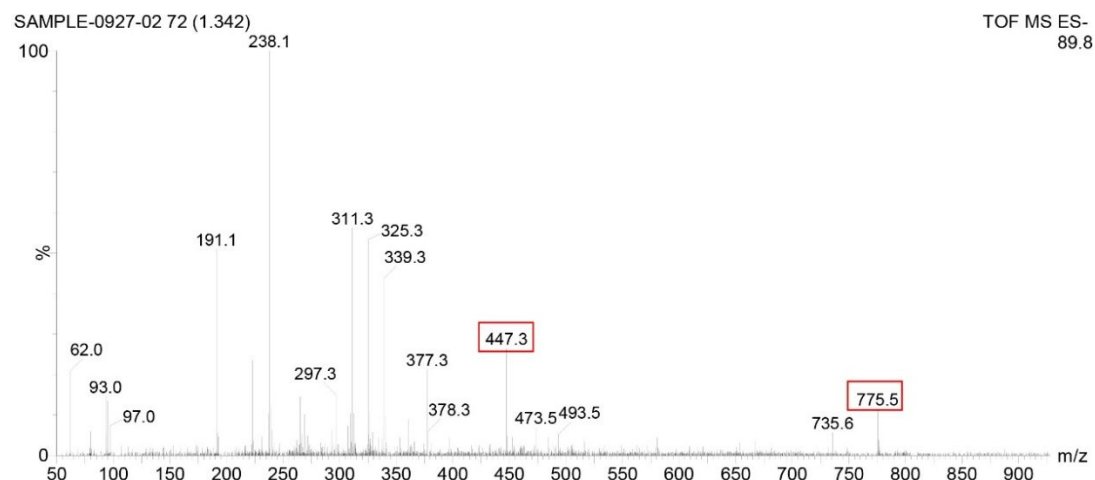
**Figure S7.** Image showing colour change after addition of sufficient  $\text{Na}_2\text{S}_2\text{O}_4$  into Nano-azo



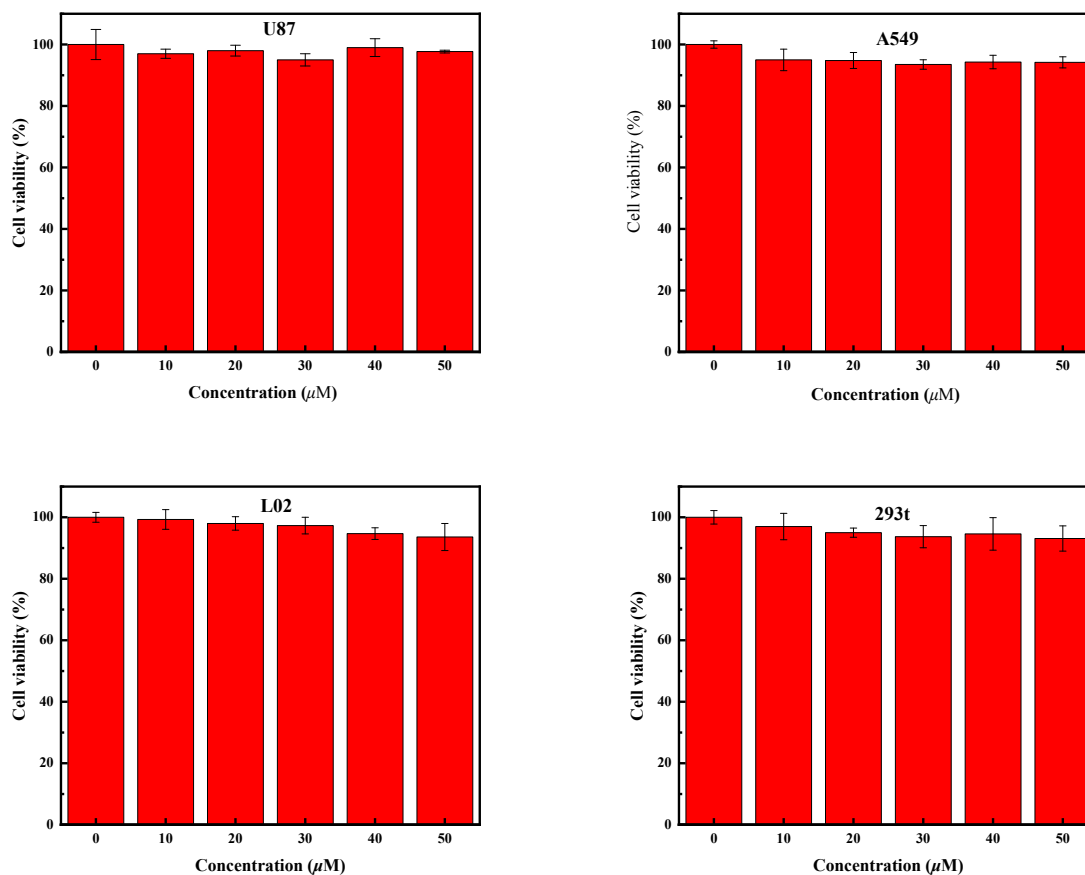
**Figure S8.** Plot of fluorescence intensity at 735 nm against the concentration of  $\text{Na}_2\text{S}_2\text{O}_4$



**Figure S9.** Fluorescence intensity after NRh-Nano was incubated with different substrates

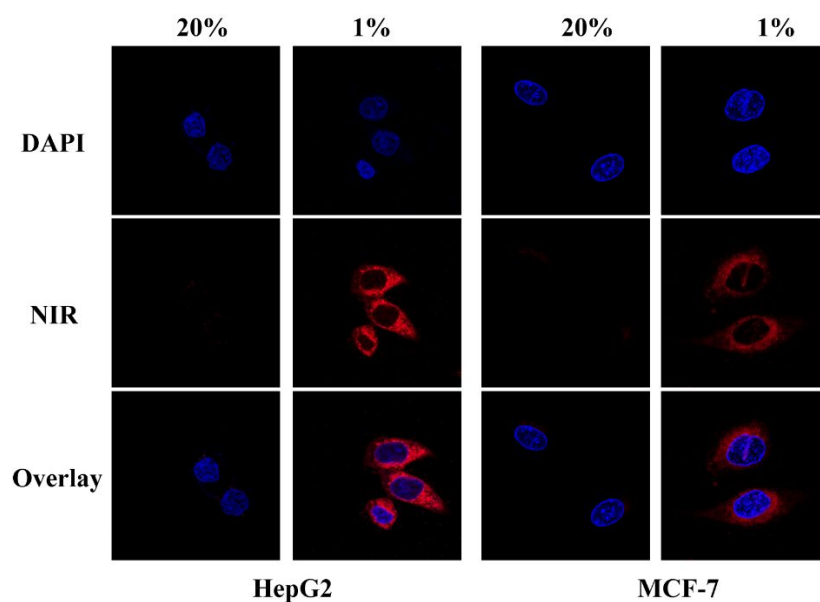


**Figure S10.** Mass spectra of NRh-Azo After reaction

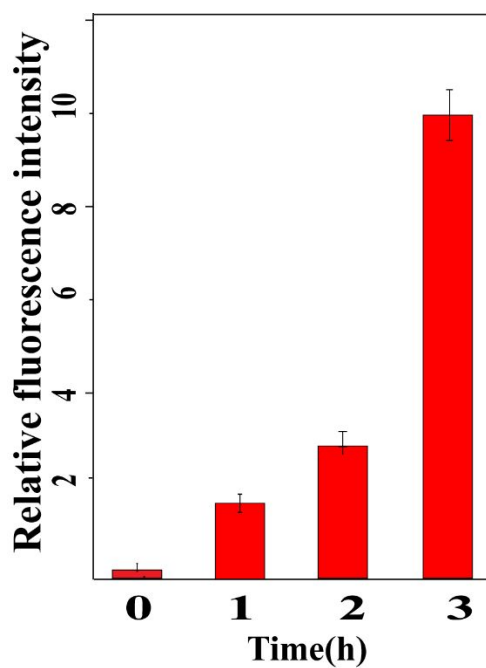


**Figure S11.** MTT assay for estimating cell viability (%) of U87, A549, L02, 293t cells treated with various concentrations of Nano-Azo (0-50  $\mu\text{M}$ ) after 24 h incubation.

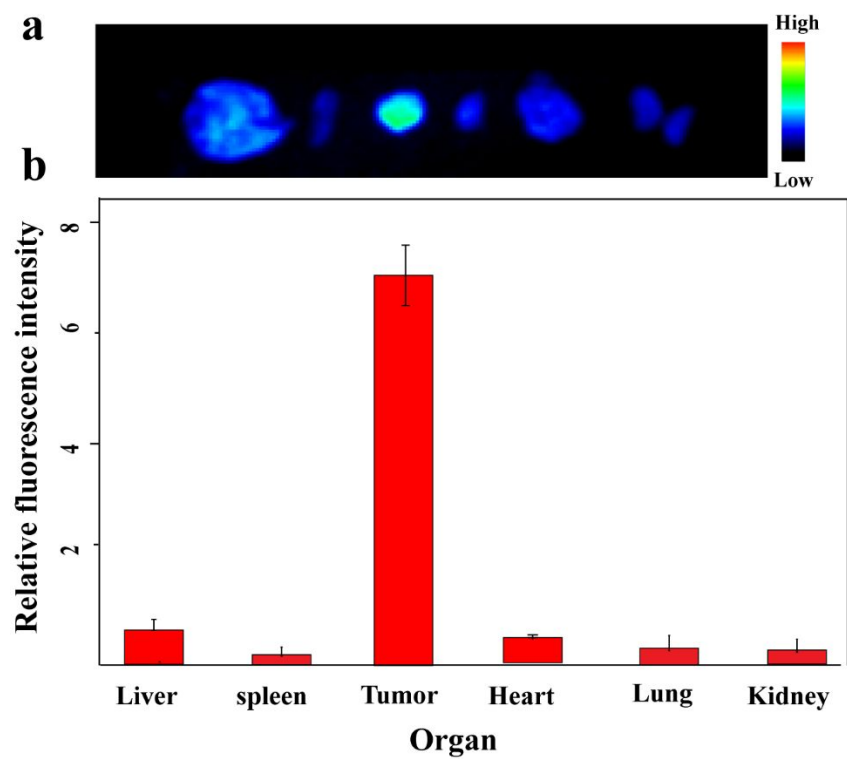




**Figure S12.** Confocal fluorescence images of HepG2 and MCF-7 cells under different oxygen concentrations (20%, 1% O<sub>2</sub>)



**Figure S13.** Relative pixel intensity of fluorescence image in Figure 5b.



**Figure S14.** (a) *Ex vivo* imaging of organs and tumor tissue. Left to right: liver, spleen, tumor, heart, lung, kidney. (b) Relative fluorescence intensity of image in a