Supporting Information

Monitoring Nitric Oxide in Subcellular Compartments by Hybrid Probe Based on Rhodamine Spirolactam and SNAP-tag

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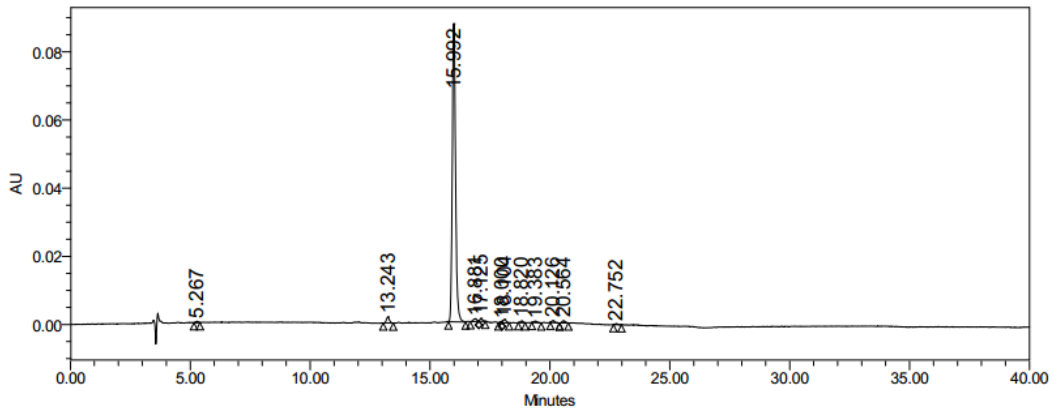
Supplementary Figures



	RT	Area	% Area	Height
1	5.267	2663	0.30	365
2	13.243	15725	1.76	1809
3	15.992	827444	92.43	88138
4	16.881	8775	0.98	781
5	17.125	6397	0.71	861
6	18.000	1968	0.22	453
7	18.104	9444	1.05	985
8	18.820	4119	0.46	479
9	19.383	4309	0.48	415
10	20.126	5522	0.62	592
11	20.564	6620	0.74	624
12	22.752	2253	0.25	275

Figure S1. HPLC spectrum of **TMR-NO-BG**

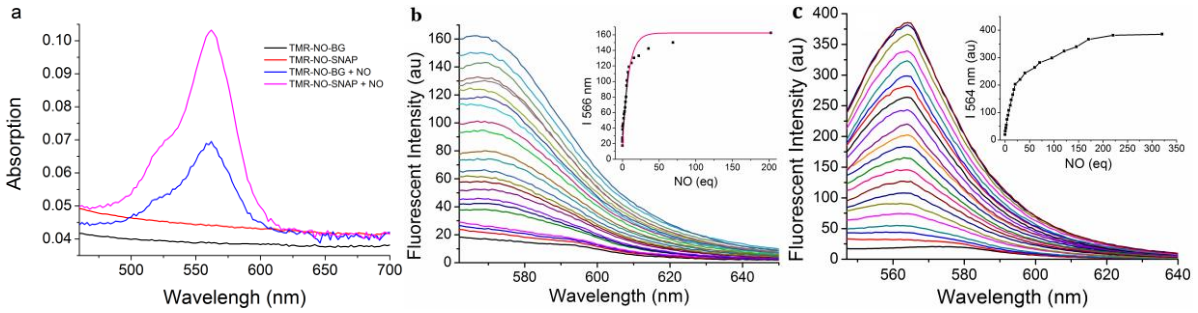


Figure S2. Spectra of **TMR-NO-BG** and **TMR-NO-SNAP**. (a) Absorption spectra of **TMR-NO-BG** (10 μM in PBS at PH 7.4) TMR-NO-SNAP (10 μM) before and after the addition of NO (200 equiv). (b) Fluorescence spectra of **TMR-NO-BG** (0.5 μM in 20 mM HEPES at PH 7.2) excited at 535 nm upon the addition of NO from 0 to 200 equiv. Inset: the response of fluorescent intensity at 566 nm upon the addition of NO. (c) Fluorescence spectra of **TMR-NO-SNAP** (0.5 μM in 20 mM HEPES at PH 7.2) upon the addition of NO from 0 to 320 equiv. Inset: the response of fluorescent intensity at 564 nm upon the addition of NO.

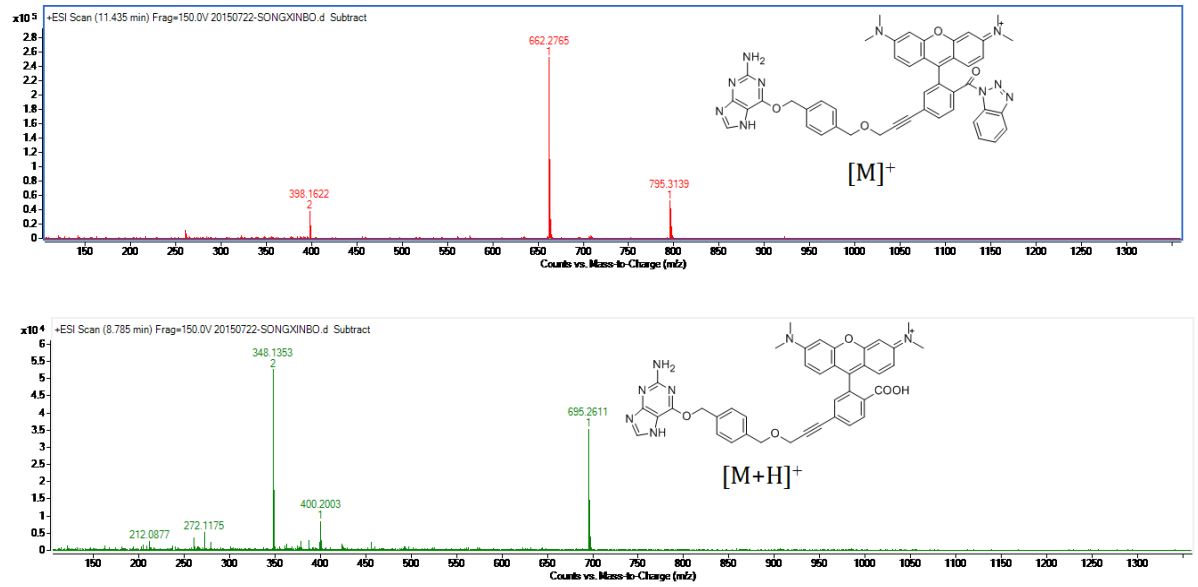


Figure S3. LC-MS spectra of production of **TMR-NO-BG** with NO.

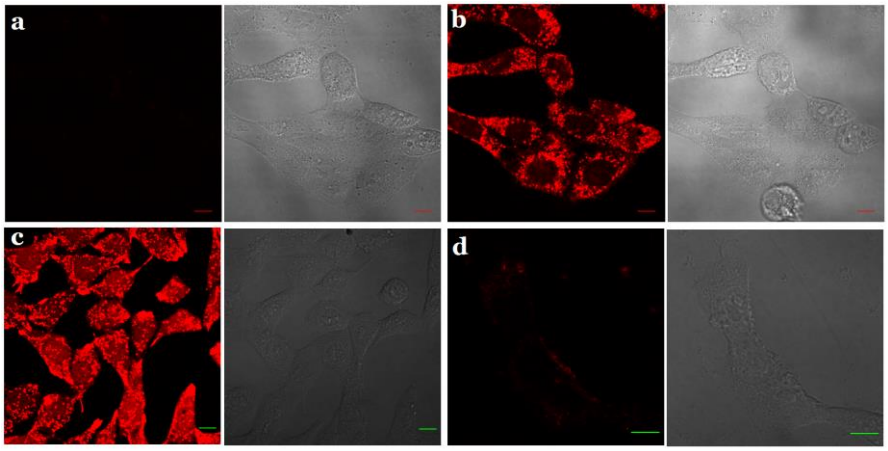


Figure S4. Fluorescent imaging of nontransfected COS-7 cells labeled with **TMR-NO-BG**. Cells were stained with **TMR-NO-BG** (5 μM) for 30 min (a, b, d) and 60 min (c). (a, b, c) Cells were washed with PBS. Fluorescent imagings were collected before (a) and after (b, c) NO solutions (20 equiv) were added. (d) After the washing step and incubation time (2 h), NO solutions (20 equiv) were added. (λ_{ex}, 559 nm; λ_{em}, 575-675 nm).

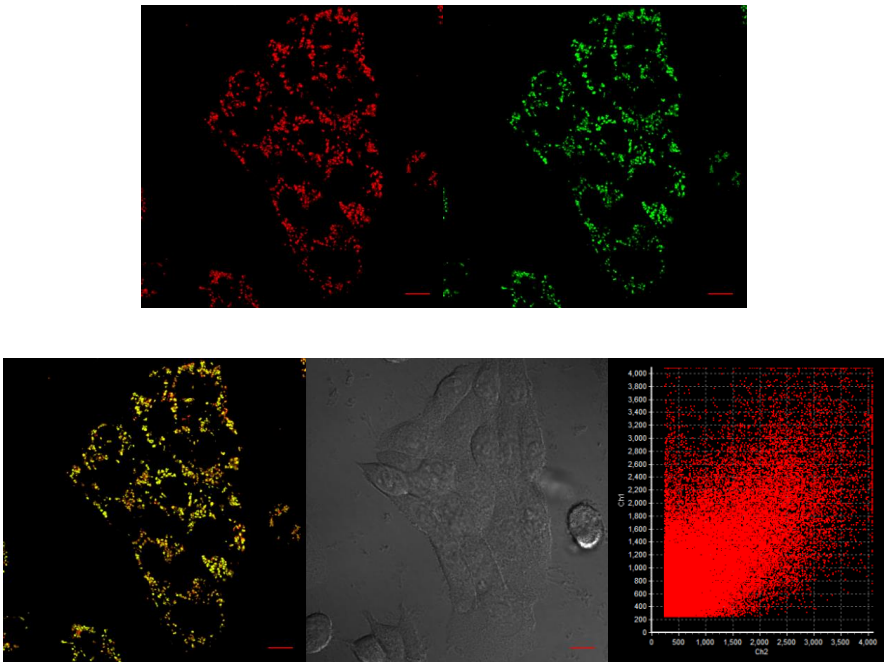


Figure S5. Colocalization of **TMR-NO-BG** labeled (5 μM for 45 min) COS-7 cells stable expressed mitochondrial targeted SNAP-tag. Fluorescent imaging was collected after NO solution (20 equiv) was added (λ_{ex}, 559 nm; λ_{em}, 575-620 nm). MitoTracker Deep Red FM is used for colocalization (0.2 μM for 10 min, λ_{ex}, 635 nm; λ_{em}, 655-755 nm).

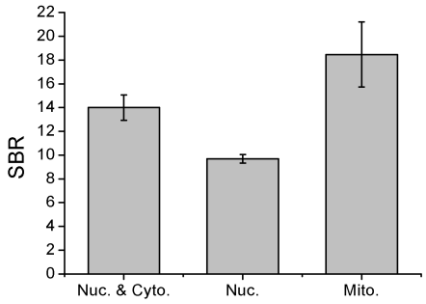


Figure S6. The signal-to-background ratio (SBR) of in different subcellular compartments of COS-7 cells labeled with **TMR-NO-BG**. The SBR values were calculated from the average fluorescent intensities of the labeled cells and background area.

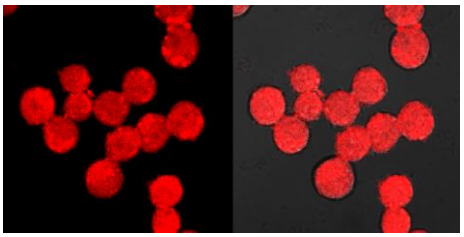


Figure S7. Fluorescence imaging of RAW264.7 cells express diffused SNAP-tag stained with **TMR-NO-BG**. NO solution (20 equiv) was added to light on the probe to confirm the location. (λ_{ex}, 559 nm; λ_{em}, 575-675 nm).

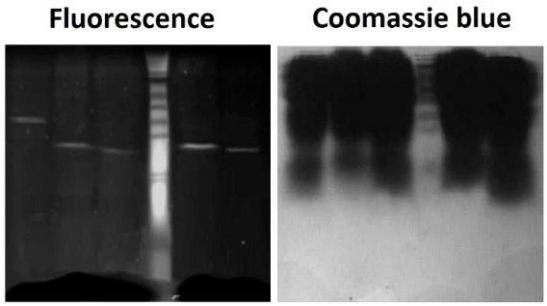
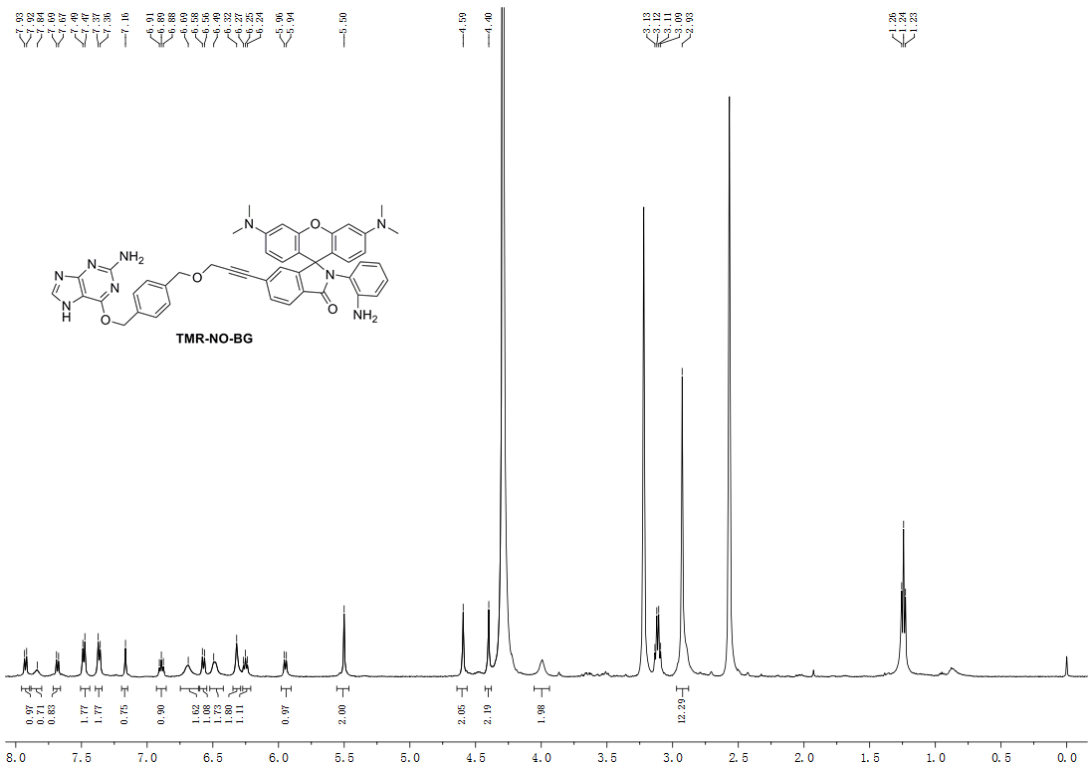


Figure S8. SDS-PAGE analysis of **TMR-NO-BG** labeled cells. Fluorescence (left) and coomassie blue stained (right) imaging of the same gel.


7.93
7.92
7.84
7.69
7.67
7.49
7.47
7.37
7.36
7.16
6.91
6.89
6.88
6.69
6.58
6.56
6.49
6.32
6.27
6.25
6.24
5.96
5.94
5.90
4.59
4.40
3.13
3.12
3.11
3.09
2.93
1.26
1.24
1.23
NH2
NH2
TMR-NO-BG
0.97
0.71
0.83
1.77
1.77
0.75
0.90
1.62
1.08
1.73
1.89
1.11
0.97
2.00
2.05
2.19
1.98
12.29
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0