Electronic Supplementary Information:

Modulating the GSH/Trx Selectivity of a Fluorogenic Disulfide-based Thiol Sensor to Reveal Diminished GSH Levels under ER Stress

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S1. Synthesis & Characterization

Scheme S1. Synthetic route to **ER-Ref** and **ER-Naph**. (a) HNO$_3$, H$_2$SO$_4$, 1,4-dioxane; (b) Raney Ni, H$_2$ (g), methanol, THF; (c) amyl nitrite, NaN$_3$, DMF, water; (d) propargyl amine, ethanol; (e) phosgene, DIPEA, 2,2'-dithiodiethanol, THF; (f) 6, condition A for **ER-Ref**, or 7, condition B for **ER-Naph**. Condition A = CuSO$_4$/THPTA (5 mol %, 1:5 ratio), sodium ascorbate (25 mol %), THF/phosphate buffer (0.1 M, pH 7.0) (2:1 = v/v); Condition B = CuSO$_4$/TBTA (1:1 ratio), sodium ascorbate, THF/t-BuOH/water (1:1:1 = v/v/v). TBTA = tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine, THPTA = tris[(3-hydroxypropyl-1H-1,2,3-triazol-4-yl)methyl]amine.
Reagents were purchased from commercial sources and used without further purification. $^1$H, $^{13}$C NMR, HH COSY, CH HSQC spectra were measured on Bruker Avance III 600 NMR spectrometer ($^1$H = 600 MHz) equipped with a PABBO BB-1H Z GRD probe head or measured on JEOL JNM-AL300 spectrometer ($^1$H = 300 MHz). Chemical shifts were reported as in units of parts per million (ppm), and J-values are in Hz. FAB-MS spectra were measured on JEOL JMS-700W.

S1-1. Synthesis of Azido-glibenclamide

![Scheme S1-1. Molecular structures of glibenclamide derivatives and the marking of aromatic rings (a, b) and hydrogens of amide/sulfonylurea (α, β, γ).](image)

Scheme S1-1. Molecular structures of glibenclamide derivatives and the marking of aromatic rings (a, b) and hydrogens of amide/sulfonylurea (α, β, γ).

Compound 1 (glibenclamide, TCI G0382)

$^1$H, $^{13}$C NMR of glibenclamide was measured for the next step NMR characterization.

$^1$H NMR (DMSO-$d_6$, 300 MHz): $\delta$ 10.30 (s, NH$_\beta$, 1H), 8.25 (t, NH$_\alpha$, J = 5.5 Hz, 1H), 7.82 (d, H$_b$, J = 8.4 Hz, 2H), 7.62 (d, H$_a$, J = 2.6 Hz, 1H), 7.49 (dd, H$_a$, J = 8.8, 2.6 Hz, 1H), 7.47 (d, H$_b$, J = 8.4 Hz, 2H), 7.14 (d, H$_a$, J = 8.8 Hz, 1H), 6.32 (d, NH$_\gamma$, J = 7.3 Hz, 1H), 3.78 (s, O-CH$_3$, 3H), 3.53 (m, NH$_a$-CH$_2$-, 2H), 3.2 (br, overlapped with H$_2$O, 1H), 2.92 (t, NH$_a$-CH$_2$CH$_2$Ar, J = 6.96 Hz, 2H), 1.6-1.0 (m, 10H) ppm; $^{13}$C NMR (DMSO-$d_6$, 75 MHz): $\delta$ 163.5, 155.6, 150.4, 145.1, 138.1, 131.4, 129.5, 129.2, 127.2, 124.7, 124.3, 114.0, 56.1, 48.0, 40.1, 34.6, 32.2, 24.9, 24.1 ppm.
Compound 2
Concentrated H$_2$SO$_4$ (10 mL) was added to concentrated HNO$_3$ (10 mL) in an ice bath. To the mixture 1 (3.0 g, 6.3 mmol) as a suspension in 1.4-dioxane (4 mL) was slowly added. After 30 min stirring at 0 °C, the mixture was warmed to room temperature and stirred for 1 hour (Caution: Keep the reaction scale and temperature. If not, the overheated reaction mixture will boil vigorously). The reaction mixture was partitioned between water and ethyl acetate. The organic layer was washed with water (× 2), saturated NaHCO$_3$ (× 2), brine and dried over anhydrous MgSO$_4$. The reaction mixture was dissolved in hot ethanol (100 mL) and slowly cooled. The sonication of the mixture resulted in an emulsion-like precipitate. The resulting precipitate was collected by filtration to yield 2 as a white solid (370 mg, 0.71 mmol, yield 11 %). TLC (hexane:ethyl acetate = 1:2): $R_f$ (1) = 0.23, $R_f$ (2) = 0.33.

$^1$H NMR (DMSO-d$_6$, 300 MHz): $\delta$ 10.29 (s, NH$_\beta$, 1H), 8.68 (t, NH$_\alpha$, $J = 5.5$ Hz, 1H), 8.14 (d, H$_a$, $J = 2.6$ Hz, 1H), 7.81 (d, H$_{10}$, $J = 8.0$ Hz, 2H), 7.70 (d, H$_a$, $J = 2.6$ Hz, 1H), 7.49 (d, H$_a$, J = 8.4 Hz, 2H), 6.32 (d, NH$_T$, $J = 7.7$ Hz, 1H), 3.58 (s, O-CH$_3$, 3H), 3.55 (m, overlapped with O-CH$_3$, 2H), 3.2 (br, overlapped with H$_2$O, 1H), 2.94 (t, NH$_\alpha$-CH$_2$CH$_2$Ar, $J = 6.8$ Hz, 2H), 1.6-1.0 (m, 10H) ppm; $^{13}$C NMR (DMSO-d$_6$, 75 MHz): $\delta$ 163.3, 150.3, 148.2, 144.9, 144.4, 138.2, 133.8, 132.8, 129.2, 127.4, 127.2, 125.3, 62.9, 48.0, 34.3, 32.2, 24.9, 24.1 ppm; HR-MS (FAB+): $m/z$ calculated 538.1289 for C$_{23}$H$_{27}$ClN$_4$O$_7$S, observed 539.1369 for [M + H]$^+$. 
Fig. S1-1. $^1$H NMR (DMSO-d$_6$, 300 MHz) of 2

Fig. S1-2. $^{13}$C NMR (DMSO-d$_6$, 75 MHz) of 2
Compound 3

Raney Nickel (ca. 0.20 g, wet in water) was added to 2 (0.37 g, 0.71 mmol) in anhydrous THF (10 mL). H₂ was bubbled by balloon until 2 disappeared on TLC. The reaction mixture was filtered on a celite pad and Raney nickel was filtered off. After removal of the solvents, the product was characterized without further purification to yield 3 as a white solid (0.35 g, 0.68 mmol, yield 95%).

¹H NMR (DMSO-d₆, 300 MHz): δ 10.16 (s, NHβ, 1H), 8.29 (t, NHα, J = 4.7 Hz, 1H), 7.80 (d, Hb, J = 7.7 Hz, 2H), 7.46 (d, Hb, J = 7.7 Hz, 2H), 6.74 (s, Ha, 1H), 6.56 (d, Ha, 1H), 6.29 (d, NHγ, J = 7.3 Hz, 1H), 5.39 (s, 2H), 3.51 (m, 2H), 3.44 (s, 3H), 2.92 (t, J = 6.6 Hz, 2H), 1.6-1.0 (m, 11H) ppm; ¹³C NMR (DMSO-d₆, 75 MHz): δ 164.9, 150.4, 145.1, 143.4, 142.2, 138.1, 130.4, 129.1, 127.8, 127.2, 115.2, 114.6, 59.9, 48.0, 34.5, 32.2, 24.9, 24.1 ppm; HR-MS (FAB+): m/z calculated 508.1547 for C₂₃H₂₀ClIN₄O₅S, observed 509.1615 for [M + H]⁺.
Fig. S1-4. $^1$H NMR (DMSO-d$_6$, 300 MHz) of 3

Fig. S1-5. $^{13}$C NMR (DMSO-d$_6$, 75 MHz) of 3
Compound 4

3 (0.15 g, 0.29 mmol) as dissolved in 5 mL of dimethylformamide (DMF). To this solution was added 5 mL of water, and the DMF concentration was increased until a clear solution was obtained. The solution was acidified by the addition of 1 mL of concentrated sulfuric acid followed by 0.2 mL of amyl nitrite. After the solution was stirred and cooled for a further 30 min at 4 °C, sodium azide (0.20 g in 5 mL of water) was added. The mixture was stirred and cooled for a further 30 min, and then a saturated solution of sodium chloride was added until a volume of 20 mL was reached. The product was characterized without further purification to yield 4 as a yellow solid (0.15 g, 0.28 mmol, yield 95 %).

$^1$H NMR (DMSO-$d_6$, 600 MHz): $\delta$ 10.25 (s, NH$_\beta$, 1H), 8.43 (t, NH$_\alpha$, J = 5.58 Hz, 1H), 7.76 (d, H$_b$, J = 8.3 Hz, 2H), 7.43 (d, H$_b$, J = 8.1 Hz, 2H), 7.28 (d, H$_a$, J = 2.6 Hz, 1H), 7.16 (d, H$_a$, J = 2.5 Hz, 1H), 6.27 (d, NH$_\gamma$, J = 7.6 Hz, 1H), 3.50 (s, O-CH$_3$, 3H), 3.49 (m, NH$_\alpha$-CH$_2$H$_2$, 2H), 3.20 (t, J = 3.84 Hz, 1H), 2.88 (t, NH$_\alpha$-CH$_2$CH$_2$, J = 6.96 Hz, 2), 1.58-1.0 (m, 10H) ppm; $^{13}$C NMR (DMSO-$d_6$, 150 MHz): $\delta$ 163.9, 150.4, 148.0, 145.0, 138.2, 134.4, 132.5, 129.3 (Ar$_\alpha$-
C), 128.1, 127.3 (Arα-C), 124.9 (Arβ-C), 122.0 (Arγ-C), 62.1 (O-CH3), 48.0 (NHγ-CH), 34.4 (NHα-CH2CH2), 32.3 (CyHex-C), 25.0 (CyHex-C), 24.2 (CyHex-C) ppm; HR-MS (FAB+): m/z calculated 534.1452 for C23H27ClN6O5S, observed 535.1529 for [M + H]+.

Caution! Workup of reactions involving inorganic azide should avoid acid, as HN3 is volatile, explosive, and highly toxic.

Fig. S1-7. ¹H NMR (DMSO-d6, 600 MHz) of 4
Fig. S1-8. $^{13}$C NMR (DMSO-d$_6$, 150 MHz) of 4

<table>
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<th>Ion mode</th>
<th>FAB+</th>
<th>Inlet</th>
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</tr>
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<td>RT</td>
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<tr>
<td>Mass Tolerance</td>
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<td>Unsaturation</td>
<td>0.0 – 100.0</td>
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</tbody>
</table>

Elements: C 1500.1, H 1500.0, Cl 20.0 (35Cl 20.0, 37Cl 20.0), N 84.0, O 76.0, S 20.0

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<tr>
<th>Observed m/z</th>
<th>Int%</th>
<th>Error (ppm/amu)</th>
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<td>13.5</td>
<td>C23H23Cl2NS4O5 S</td>
</tr>
</tbody>
</table>

Fig. S1-9. HR MS of 4
Fig. S1-10. HH COSY (homonuclear correlation spectroscopy) of 4
Fig. S1-11. HSQC (heteronuclear single quantum coherence) of 4
S1-2. Synthesis of ER-Naph

4-Amino-N-(2-propynyl)-1,8-naphthalimide (6)

Compound 6 was prepared from 4-amino-1,8-naphthalic anhydride by the Wolfbeis’s procedure and the spectra of $^1$H & $^{13}$C NMR are consistent with the literature.$^1$

$^1$H NMR (DMSO-$d_6$, 300 MHz): $\delta$ 8.72 (d, $J = 7.5$ Hz, 1H), 8.53 (d, $J = 6.4$ Hz, 1H), 8.29 (d, $J = 8.4$ Hz, 1H), 7.76 (t, $J = 7.9$ Hz, 1H), 6.69 (d, $J = 8.4$ Hz, 1H), 4.80 (d, $J = 2.5$ Hz, 2H), 3.15 (t, $J = 2.4$ Hz, 1H) ppm; $^{13}$C NMR (DMSO-$d_6$, 75 MHz): $\delta$ 163.0, 162.0, 153.1, 134.2, 131.3, 129.7, 124.0, 121.4, 119.3, 108.3, 106.9, 79.9, 72.4, 28.6 ppm.

Compound 7

To 6 (20 mg, 0.080 mmol) in 30 mL of THF was added DIPEA (97 $\mu$L, 0.56 mmol) dropwise. To the reaction mixture was added phosgene (300 $\mu$L, 0.42 mmol, 15 wt.% in toluene). The solution was stirred for 4 h under nitrogen gas. After removal of unreacted phosgene gas by rotary evaporator, the residual was co-evaporated with CH$_2$Cl$_2$ (10 mL) and THF (10 mL). The evaporated fraction was neutralized in a NaOH bath. To the residual was added a solution of 2,2'-dithiodiethanol (48 $\mu$L, 0.40 mmol) in CH$_2$Cl$_2$/THF (v/v, 1:1). The reaction mixture was stirred overnight. The solvent was evaporated off, at which point CH$_2$Cl$_2$ (30 mL) and water (30 mL) were added, and the organic layer was collected. The CH$_2$Cl$_2$ layer was dried over anhydrous MgSO$_4$. After removal of the solvents, the crude product was purified over silica gel using hexane/ethyl acetate (1:2, v/v) to yield the desired 7 as a yellow solid (14 mg, 0.033 mmol, yield: 40%).

$^1$H NMR (DMSO-$d_6$, 300 MHz): $\delta$ 10.41 (s, 1H), 8.70 (d, $J = 8.4$ Hz, 1H), 8.49 (t, $J = 8.1$ Hz, 2H), 8.17 (d, $J = 8.2$ Hz, 1H), 7.83 (t, $J = 7.9$ Hz, 1H), 4.75 (d, $J = 2.4$ Hz, 2H), 4.45 (t, $J = 6.4$ Hz, 2H); 3.64 (t, $J = 6.4$ Hz, 2H), 3.14 (t, $J = 2.4$ Hz, 1H), 3.09 (t, $J = 6.4$ Hz, 2H), 2.84 (t, $J = 6.4$ Hz, 2H) 2.49 (t, $J = 1.8$ Hz, 1H) ppm; $^{13}$C NMR (DMSO-$d_6$, 75 MHz): $\delta$ 162.7,
162.1, 153.9, 141.1, 132.1, 128.3, 126.5, 123.9, 121.8, 118.4, 116.6, 79.4, 73.0, 63.0, 59.5, 59.4, 41.1, 36.7, 29.0 ppm; HR-MS (FAB+): m/z calculated 430.0657 for \( \text{C}_{20}\text{H}_{18}\text{N}_2\text{O}_5\text{S}_2 \), observed 431.0737 for \([\text{M} + \text{H}]^+\).

Fig. S1-12. \(^1\)H NMR (DMSO-d\(_6\), 300 MHz) of 7

Fig. S1-13. \(^{13}\)C NMR (DMSO-d6, 75 MHz) of 7
Fig. S1-14. HR-MS (FAB+) of 7

Scheme S1-2. Molecular structures of **ER-Ref** and **ER-Naph** and the marking of aromatic rings (a, b, c, d) and hydrogens of amide/sulfonyleurea (α, β, γ).
ER-Ref

4 (0.070 g, 0.13 mmol) was dissolved in a 5 mL (1:1, v/v) cosolvent of THF/phosphate buffer (0.1 M, pH 7.0) and 6 (0.033 g, 0.13 mmol) was added. CuSO$_4$/THPTA$^2$ (1:5 ratio) and sodium ascorbate were added to reach the final concentrations of 1 mM for Cu$^{2+}$ and 5 mM for sodium ascorbate. After complete addition, the reaction mixture was maintained for 1 h at room temperature, and the mixture was poured into water and extracted with ethyl acetate. The organic phase was separated and dried over MgSO$_4$. The solvent was removed by rotary evaporation, and product were purified by silica gel column chromatography using dichloromethane/methanol (10:1) to yield the desired compound ER-Ref (0.050 g, 0.063 mmol, yield: 46%).

$^1$H NMR (DMSO-d$_6$, 600 MHz): δ 10.3 (s, NH$\beta$, 1H), 8.64 (td, NH$_n$+ H$_c$, 2H), 8.46 (d, H$_c$, J = 8.46 Hz, 1H), 8.36 (s, N$_3$-H, 1H), 8.22 (d, H$_d$, J = 8.40 Hz, 1H), 7.82 (m, H$_{a+b}$, 3H), 7.68 (t, H$_c$, J = 7.44 Hz, 1H), 7.57 (d, H$_n$, J = 2.70 Hz, 1H), 7.51 (m, H$_{b}$+NH$_2$, 4H), 6.86 (d, H$_a$, J = 8.40 Hz, 1H), 6.31 (d, H$_d$, J = 7.70 Hz, 1H), 5.37 (s, 2H), 3.56 (m, NH$\alpha$-CH$_2$, 2H), 3.38 (s, O-CH$_3$, 3H), 3.24 (m, 1H), 2.94 (t, NH$_o$-CH$_2$-CH$_2$, J = 7.02 Hz), 1.61-1.03 (m, 10H) ppm; $^{13}$C NMR (DMSO-d$_6$, 75 MHz): δ 163.8, 163.6, 162.6, 152.9, 149.1, 144.7, 144.0, 134.1, 132.7, 131.3, 131.2, 129.8, 129.7, 129.5, 129.1, 127.5, 127.2, 125.0, 124.0, 121.7, 119.3, 108.2, 107.3, 61.9, 48.0, 34.6, 34.4, 32.2, 30.4, 24.9, 24.1 ppm; HR-MS (FAB+): m/z calculated 784.2194 for C$_{38}$H$_{37}$ClN$_8$O$_7$S, observed 785.2267 for [M + H]$^+$. 

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Fig. S1-15. $^1$H NMR (DMSO-d$_6$, 600 MHz) of ER-Ref

Fig. S1-16. $^{13}$C NMR (DMSO-d$_6$, 75 MHz) of ER-Ref
ER-Naph

4 (18 mg, 0.034 mmol) was dissolved in a 2 mL (1:1, v/v) cosolvent of THF/t-BuOH, and then 7 (10 mg, 0.023 mmol) was added to the solution. A click cocktail (TBTA (0.62 mg, 0.0012 mmol), CuSO$_4$·5H$_2$O (0.29 mg, 0.0012 mmol), and sodium ascorbate (1.2 mg, 0.0059 mmol) in 2 mL ethanol-water (1:1, v/v)) was added to the reaction mixture. The mixture was stirred for 1 day at room temperature. The solvent was removed by rotary evaporation, and the residue was purified by silica gel column chromatography using dichloromethane/methanol (30:1) to yield the desired compound ER-Naph (0.011 g, 0.011 mmol, yield: 49%).

$^1$H NMR (DMSO-d$_6$, 600 MHz): $\delta$ 10.43 (s, 1H), 10.31 (s, 1H), 8.73 (d, $J = 8.6$ Hz, 1H), 8.63 (t, $J = 5.6$ Hz, 1H), 8.55 (d, $J = 7.3$ Hz, 1H), 8.53 (d, $J = 10.9$ Hz, 1H), 8.44 (s, 1H), 8.19 (d, $J = 8.3$ Hz, 1H), 7.87 (t, $J = 7.9$ Hz), 7.81 (dd, $J = 5.5, 1.4$ Hz, 3H), 7.57 (d, $J = 2.7$ Hz, 1H), 7.49 (d, $J = 8.3$ Hz, 2H), 6.32 (d, $J = 7.6$ Hz, 1H), 5.41 (s, 2H), 4.93 (t, $J = 5.4$ Hz, 1H).
1H), 4.45 (t, J = 6.4 Hz, 2H), 3.65 (q, J = 6.1 Hz, 2H), 3.55 (q, J = 6.6 Hz, 2H), 3.10 (t, J = 6.4 Hz, 2H), 2.93 (t, J = 7.1 Hz, 2H), 2.85 (t, J = 6.4 Hz, 2H), 2.08 (s, 1H) ppm; 13C NMR (DMSO-d6, 75 MHz): δ 163.9, 163.3, 162.7, 153.9, 150.4, 149.2, 145.0, 143.7, 143.6, 140.9, 138.2, 136.2, 132.8, 131.9, 131.3, 131.1, 129.8, 129.6, 129.3, 128.7, 128.5, 128.0, 127.7, 127.6, 127.3, 126.5, 125.1, 124.2, 124.1, 122.2, 118.6, 117.1, 63.0, 62.0, 59.4, 52.7, 48.0, 41.1, 36.7, 34.4, 32.2, 24.9, 24.1 ppm; HR-MS (FAB+): m/z calculated 964.2109 for C43H45ClN8O10S3, observed 965.2197 for [M + H]+

Fig. S1-18. 1H NMR (DMSO-d6, 600 MHz) of ER-Naph
Fig. S1-19. $^{13}$C NMR (DMSO-d$_6$, 75 MHz) of **ER-Naph**

Fig. S1-20. HR-MS (FAB+) of **ER-Naph**
S2. *In vitro* Photophysical Characterization

UV/vis spectra were measured on JASCO V-560 UV/vis spectrophotometer at ambient temperature. A cell with a 1 cm path length (Quarts SUPRASIL 105B-QS) was used for measuring. Each sample was prepared from a serial dilution of sample stock solution (1.0 mM in DMSO). The concentration of final solution for absorption measurement was 10 μM (1% DMSO) in 1× PBS buffer (pH 7.4).

Emission spectra were measured at ambient temperature on RF5301PC (Shimadzu). Cells with a 1 cm path length were used for measuring. Each sample was prepared from a serial dilution of sample stock solution (1.0 mM in DMSO). The concentration of final solution for fluorescence measurement was 1.0 μM (0.1% DMSO) in 1× PBS buffer (pH 7.4). Slit width: excitation = 3 nm, emission = 5 nm.

All data related with GSH were acquired after 3 h incubation adding GSH. The samples were incubated in Eppendorf Thermomixer (1,200 rpm, 37 °C).
Figure S2-1. UV/vis absorption (A) and fluorescence (B) spectra of ER-Ref and ER-Naph in PBS buffer (pH 7.4). For (A), [probe] = 10 μM, $\lambda_{\text{max}}^{\text{abs}} = 436$ nm of ER-Ref, $\lambda_{\text{max}}^{\text{abs}} = 381$ nm of ER-Naph. For (B), [probe] = 1.0 μM, $\lambda_{\text{max}}^{\text{em}} = 545$ nm of ER-Ref (excited at 436 nm), $\lambda_{\text{max}}^{\text{em}} = 480$ nm of ER-Naph (excited at 381 nm). Slit width = 3/5 nm (excitation/emission).
Figure S2-2. Fluorescence spectra of **ER-Naph** upon treatment with increasing concentrations of GSH (0–5.0 mM). Inset: Change in fluorescence intensity at 545 nm as a function of GSH concentration. [*ER-Naph*] = 1.0 μM, [GSH] = 5.0 mM, excitation at 436 nm, slit width = 3/5 nm (excitation/emission). All fluorescence changes were acquired 3h after the addition of the analytes in 1X PBS buffer (pH 7.4).
S3. Time Course Measurement in Protein Extracts

Human pancreas carcinoma cell line (MIA PaCa-2) was purchased from American Type Culture Collection (ATCC) (VA. USA). The cells were cultured in DEME (Invitrogen) supplemented with 10% FCS (Invitrogen). The cells were washed with PBS and subsequently homogenized through sonication in RIPA buffer (20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM Na₂EDTA, 1 mM EGTA, 1% NP-40, 1% DMSO) with protease inhibitor cocktail for 30 min at 0 °C. The homogenate was then centrifuged at 10,000 rpm for 25 min, resulting in a clear supernatant that was transferred into a new tube. After lysis, the protein extracts were quantified by Bradford method.

Each probe was quickly mixed with GSH or protein extracts. The time-course fluorescence was measured by fluorescence plate reader (PerkinElmer VICTOR™ 3, excitation filter 450 nm (P450), emission filter BP 510-560 nm (F535)) for 3 h.

The fluorescence intensity at each time point was divided with the fluorescence intensity at 0 min and the relative fluorescence intensity was plotted.

S4. PX-12 Experiment

PX-12 from 0 to 50 µM was treated to 1X PBS buffer (pH 7.4) which included 200 µg of the MIA PaCa-2 protein extract, and the mixture was incubated for 24 h at 37 °C. incubator. After a day, each probe (5 µM) was added and incubated for 10 min (ER-Naph) or 20 min (Mito-Naph). The fluorescence intensity of each sample was measured by following S3.

The fluorescence intensity at each point was normalized with the fluorescence intensity of probe.
S5. X-ray Structure Analysis of Trx

We used the X-ray structure of human thioredoxin (reduced form, 1.7 Å resolution) from PDB bank (PDB ID = 1ERT) and the amino acid sequence and secondary structure from UniProtKB (P10599). The electrostatic potential map was calculated with Deepview/Seiss-PdbViewer v.4.1.0. Electrostatic potential parameters use atomic partial charges and Coulomb computation mode. Electrostatic scale: from −3 (red) to +3 (blue) eV.

Amino acid sequence and secondary structure:
MVKQIESKTA/FQEALDAAGD/KLVVVDFSAT/WCGPCKMIKP/FFHSLSEKYS/NVIFLEVDVD/DCQDVASECE/VKCMPTFQFF/KKGQKVGEFS/GANKEKLEAT/INELV

β Strand/Helix/Turn

Figure S5-1. 3D ribbon models and electrostatic potential map for Trx. A) 3D ribbon model on the side view, B) 3D ribbon model on top view, C)
Figure S5-2. Trx electrostatic potential map on the top side view of Fig. S5-1 C) 90 °. A) Charge distribution surface in dotted lines preference overlapped with ribbon diagram. Cys 32 and 35 at the active center depicted as yellow. B) Charge distribution surface of Trx in filled triangles preference and **Mito-Naph** in Solid 3D view. The probe structure was energy-minimized by Spartan ’08, v1.2.0 Wavefunction, Inc. Two structures are presented on the same scale.

**S6. In Vivo Imaging**

MIA PaCa-2 cells were cultured in DEME (Invitrogen) supplemented with 10% FCS (Invitrogen). One day before imaging, cells were seeded into 24-well flat-bottomed plates. Following appropriate incubation after chemical treatment, the cells were observed under a Leica TCS-SP2 confocal fluorescence microscope, 100 × objective lenses. All cell culture media and ER-Tracker™ Red were purchased from Life Technologies (USA). Tunicamycin, brefeldin A, L-buthionine-sulfoximine (BSO), N-acetylcysteine (NAC), and PX-12 were purchased from Sigma-Aldrich (MO, USA). N-Ethylmaleimide (NEM) was purchased from Thermo Fisher Science, Inc. (USA).
Figure S6-1. Time-dependent disulfide cleavage of **ER-Naph** in Mia paca-2 cells (A) Confocal microscopy images of Mia paca-2 cells treated with **ER-Naph** (5.0 μM) for 45 min. (B) Quantification of the fluorescent intensity of cells. Values are expressed time vs. intensity before photoconversion that was given an arbitrary value of probe. The cell images were obtained using excitation wavelengths of 458 nm and emission wavelengths of BP 505–550 nm.
Figure S6-2. Colocalization experiments using **ER-Ref** and ER-Tracker Red in Mia paca-2 cells. (A) Fluorescence image after **ER-Ref** treatment (458 nm excitation and BP 505-550 nm emission filter); (B) Fluorescence image after ER-Tracker Red treatment (543 nm excitation and LP 650 nm emission filter); (C) Overlaid image of (A) and (B); (D) phase contrast image. ER-Tracker Red (0.6 μM) was treated and the Mia paca-2 cells were incubated for 10 min at 37 °C. Then **ER-Ref** (5 μM) was treated and the cells were also incubated for 10 min at 37 °C. After media exchange fluorescence images were acquired using confocal microscopy.
S7. References


2. Hong, V.; Presolski, S. I.; Ma, C.; Finn, M. G. *Angew. Chem., Int. Ed.* 2009, 48, 9879-9883. THPTA was prepared by the M. G. Finn’s procedure and the spectra of $^1$H & $^{13}$C NMR are consistent with the literature. $^1$H NMR (DMSO-$d_6$, 300 MHz) $\delta$ 8.00 (s, 1H), 4.68 (t, $J$ = 5.0 Hz, 1H), 4.40 (t, $J$ = 7.2 Hz, 2H), 3.60 (s, 2H), 3.38 (dd, $J$ = 12.0, 6.9 Hz, 3H), 1.96 (s, $J$ = 5.0 Hz, 2H) ppm; $^{13}$C NMR (DMSO-$d_6$, 75 MHz) $\delta$ 143.4, 124.1, 57.5, 47.0, 46.6, 33.0 ppm.