Supporting Information

Endogenous cysteine fluorescence monitoring and its usage for tumour demarcation

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1. Experimental section

Materials

Erastin and BSO were purchased from Aladdin. All amino acids used in this work were purchased from Sigma-Aldrich. Hanks’ Balanced Salt Solution (with Ca^{2+} and Mg^{2+} HBSS), Hematoxylin and Eosin Staining Kit, 96-well transparent polystyrene microplate and 96-well black opaque plates were purchased from Beyotime. All solvents were purified prior to use. Distilled water was used after passing through a water ultra-purification system. Hitachi F-7000 fluorescence spectrophotometer was employed to measure fluorescence spectra. Hitachi U-3900 UV-vis spectrophotometer was employed to measure UV-vis spectra. Shanhai Huamei Experiment Instrument Plants provided a PO-120 quartz cuvette (10 mm). Molecular Devices SpectraMax iD5 was used in the cellular semiquantitative fluorescent experiments. Cell and tissue sections imaging experiments were performed with a Leica DMI8 fluorescence microscope. Microtome Cryostat Microm HM525NX was used to obtain the tissue slice. Male 5-week-old BALB/c-nu mice were purchased from SPF (Beijing) Biotechnology Co., Ltd. This study was performed in strict accordance with the Chinese guidelines for the care and use of laboratory animals and was approved by the Institutional Animal Care and Use Committee of Scientific Research in Shanxi University (Taiyuan, China).

Preparation of solutions of probes and analytes

Stock solution of 2-IC\textsuperscript{1} (2 mM) was prepared in DMSO. Stock solutions of 20 mM Cys and 200 mM GSH were prepared by direct dissolution in deionized water. All chemicals used were of analytical grade.

General fluorescence spectra measurements

The detection experiments were measured in PBS (pH 7.4, 10 mM). The procedure was as follows: into a PBS solution, containing 10 μM 2-IC, an analyte sample was added. The process was monitored by fluorescence spectrometer.

Cell Culture and Imaging

The HeLa cells were grown in 1640 medium supplemented with 12% FBS and 1% antibiotics at 37 °C in humidified environment of 5% CO\textsubscript{2}. Cells were plated on a 6-well plate
with slides and allowed to adhere for 24 h. Before the experiments, cells were washed with PBS 3 times.

For the starvation experiments, HeLa cells were cultured in protein free HBSS culture medium for certain time and then incubated with 2-IC (10 μM) containing PBS for 15 min at 37 °C before imaging.

**Cellular Semiquantitative Fluorescence Detection**

HeLa cells were seeded 10⁴ per well of a 96-well black opaque plates or 96-well transparent polystyrene microplate and allowed to adhere for 24 h. Subsequently, cells were washed twice with PBS and incubated with Erastin, BSO or Compound 3² containing standard 1640 culture medium for certain time. The cells were washed thrice with PBS and further stained with 2-IC (10 μM) for 20 min at 37 °C. The resulting fluorescent intensities were measured using Molecular Devices SpectraMax iD5 plate reader (λ_ex = 453 nm, λ_em = 500 nm). The final fluorescent intensity data were further calibrated by the cell viability upon same incubation conditions.

**Ex Vivo Mouse Liver and Tumor Imaging**

Male 5-week-old BALB/c-nu mouse was sacrificed and the liver was harvested immediately. The 10 mm fresh slices were obtained and divided into two groups. The first group were stained with 2-IC (50 μM) for 20 min. The second group were incubated with Cys (200 μM) for 30 min. After washing with PBS 3 times, the slices were then stained with 50 μM 2-IC for 20 min. Fluorescent images were then obtained by the fluorescence microscope (λ_ex = 480 ± 20 nm; λ_em = 527 ± 15 nm).

For the tumor imaging experiments, 10⁶ HeLa cells were injected into male 5-week-old BALB/c-nu mouse to obtain xenograft tumor bearing mouse model which was sacrificed at 9-week-old. The tumor was immediately sectioned using surgical scalpel blade and sliced for fluorescent imaging (10 mm) and histopathological analysis (5 mm).
2. Additional figures

Figure S1. Detection mechanism of probe 2-IC (10 μM) for Cys (200 μM) in PBS and the corresponding optical properties. $\lambda_{ex} = 453$ nm, slit, 5/5 nm, 700 V.

Figure S2. Cell viabilities of HeLa cells incubated with 0-50 μM 2-IC for 6 h tested by a CCK-8 assay.
Figure S3. Cell viability results of HeLa cells treated with 1. PBS, 2. 10 μM Com3, 3. 20 μM Com3, 4. 50 μM Com3, 5. 20 μM Com3 and 40 μM NAC, 6. 20 μM Com3 and 80 μM NAC, 7. 50 μM Com3 and 40 μM NAC, 8. 50 μM Com3 and 80 μM NAC for 24 h, respectively. Error bars represent standard deviations obtained from 6 wells. *P < 0.05; x, no significant difference.
3. **Additional references**
