Supporting Information

H_2Se induces reductive stress in HepG2 cells and activates cell autophagy via regulating the redox of HMGB1 protein under hypoxia

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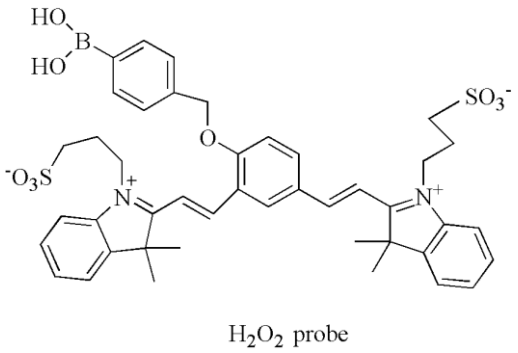
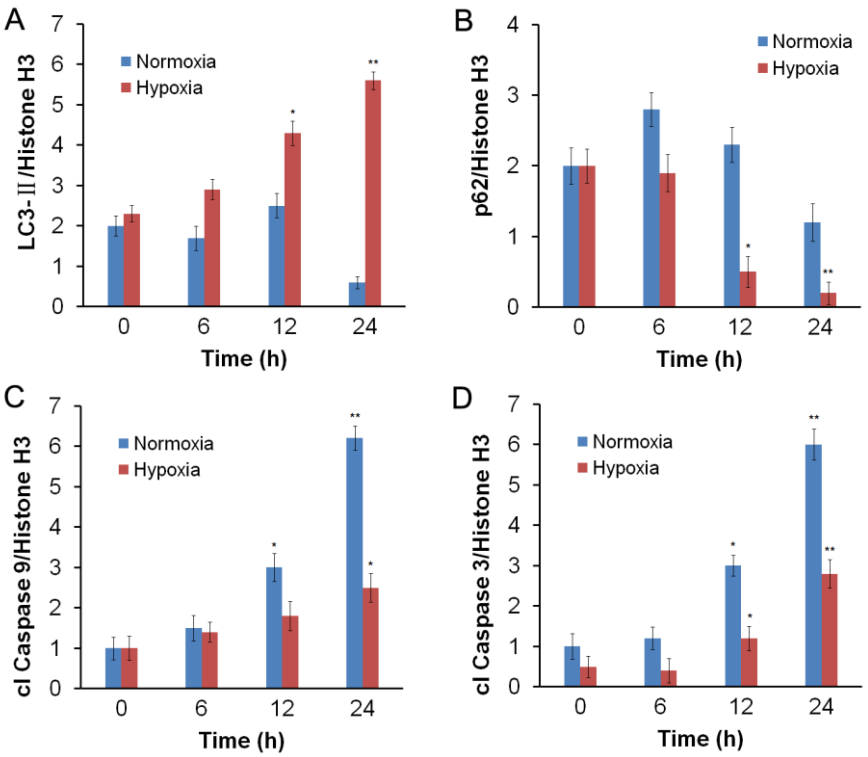
1. A H_2O_2 fluorescent probe



H_2O_2 probe

Supplementary Figure 1: The fluorescent probe for detecting H_2O_2. The synthesis and characterization of H_2O_2 probe is described in our previous study[1].

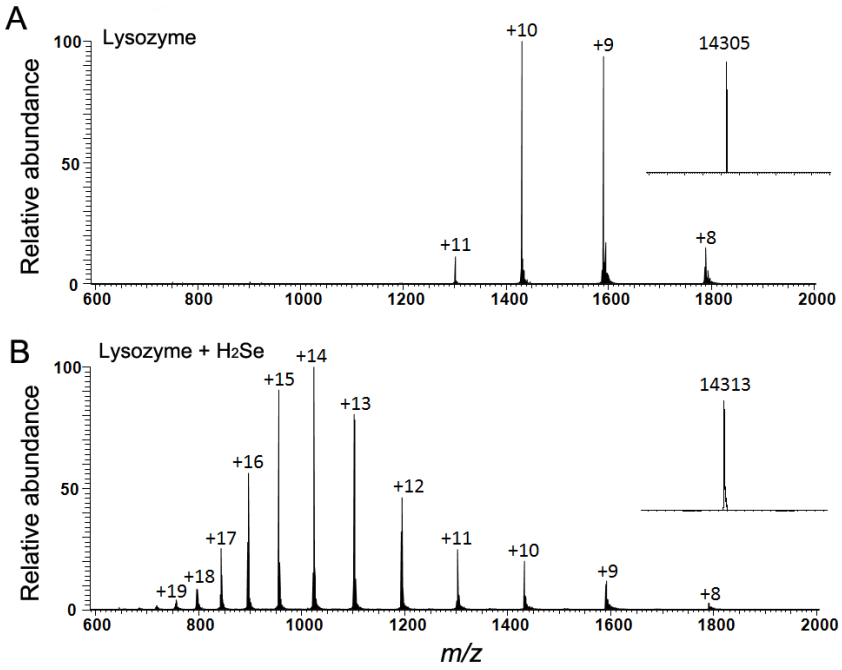
2. Protein quantification



Supplementary Figure 2: Protein quantification for Figure 4C. (A and B) Changes in the expression levels of autophagy-related proteins LC3-Ⅱ and p62. (C and D) Changes in the expression levels of apoptosis-related proteins cl Caspase 9 and cl Caspase 3. Protein bands were quantified using Image J software. (*p< 0.05, **p< 0.01, t test).

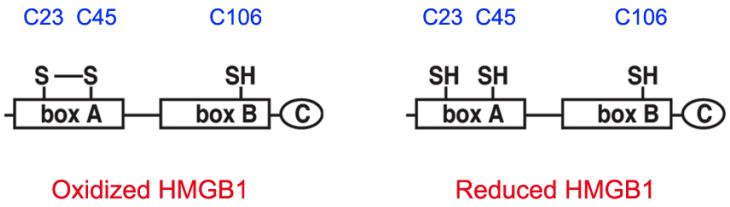
3. H_2Se can transform the disulfide bond to mercapto groups in lysozyme.

The lysozyme proteins were dissolved in sterile 18 MΩ-cm H_2O not less than 100 μg/ml, respectively, which can then be further diluted to other aqueous solutions. To examine whether H_2Se can interrupt the disulfide bond in protein, lysozyme was divided into two groups. One group was the control in which lysozyme was diluted to 200 μM in sterile H_2O, then diluted to 100 μM by MeOH: H_2O: FA = 50: 50: 1% and detected by ESSI-MS. The other group was H_2Se treatment group, in which 2 mM H_2Se was added to 200 μM lysozyme and reacted for 30 min; the mixture was then diluted to 100 μM by MeOH: H_2O: FA = 50: 50: 1% and detected by ESSI-MS.



Supplementary Figure 3: H_2Se interrupts the disulfide bond in lysozyme (containing 4 disulfide bonds). (a) 200 μM lysozyme in H_2O, then diluted to 100 μM by MeOH:H_2O:FA = 50:50:1% and detected by ESSI-MS. (b) 200 μM lysozyme plus 2 mM H_2Se in H_2O, then diluted to 100 μM by MeOH:H_2O:FA = 50:50:1% and detected by ESSI-MS.

3. Oxidized and reduced form of HMGB1



Supplementary Figure 4: The oxidized and reduced form of HMGB1.[2]

Reference

1. Pan X, Wang X, Wang L, Xu K, Kong F, Tang B. Near-Infrared Fluorescence Probe for Monitoring the Metabolic Products of Vitamin C in HepG2 Cells under Normoxia and Hypoxia. Anal Chem. 2015; 87: 7092-7.

2. Tang D, Loze MT, Zeh HJ, Kang R. The redox protein HMGB1 regulates cell death and survival in cancer treatment. Autophagy. 2010; 6: 1181-1183.