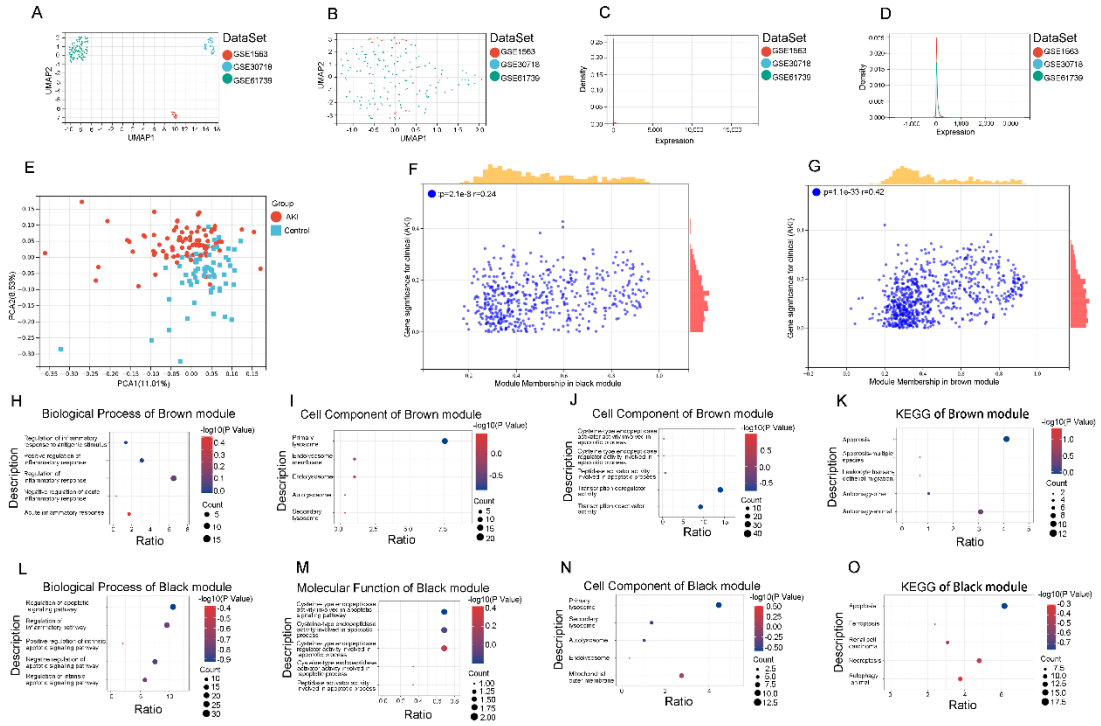
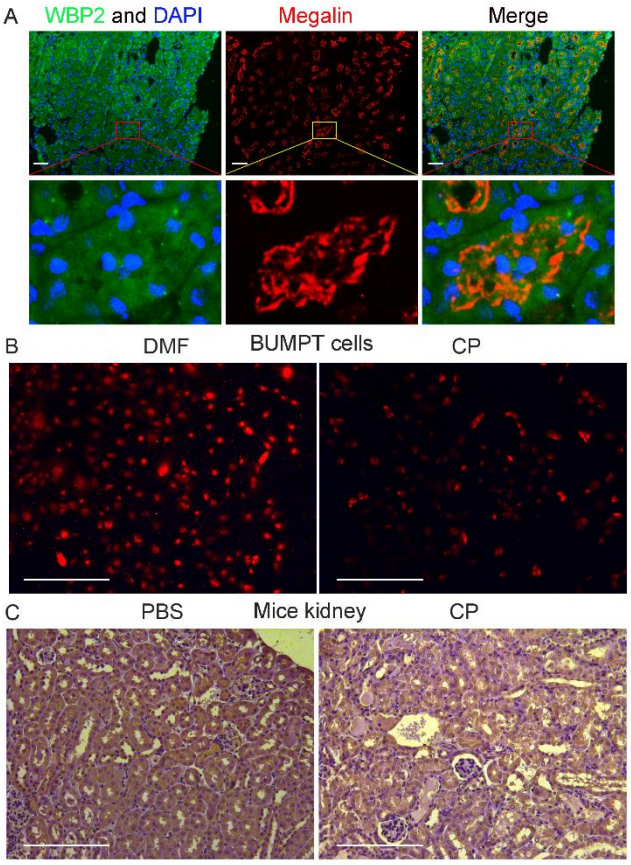


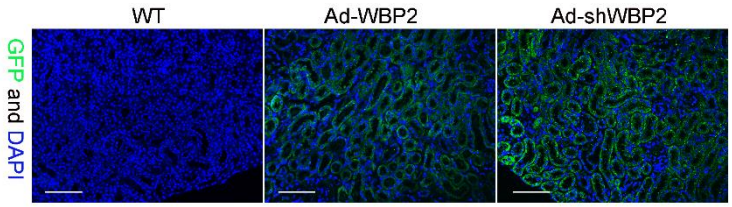
Supplemental Figure 1. Construction of the ferroptosis-related mRNA co-expression network and hub gene selection in AKI.

A - B Density plots for two datasets before batch effect correction, respectively; **C - D** UMAP plots for two datasets before batch effect correction, respectively; **E** PCA analysis of the integrated dataset; **F - G** The scatterplot of gene significance (GS) vs. MM in the co-expression Brown and Black modules; **H - I** GO and KEGG enrichment analysis results of network nodes from Brown and Black modules.



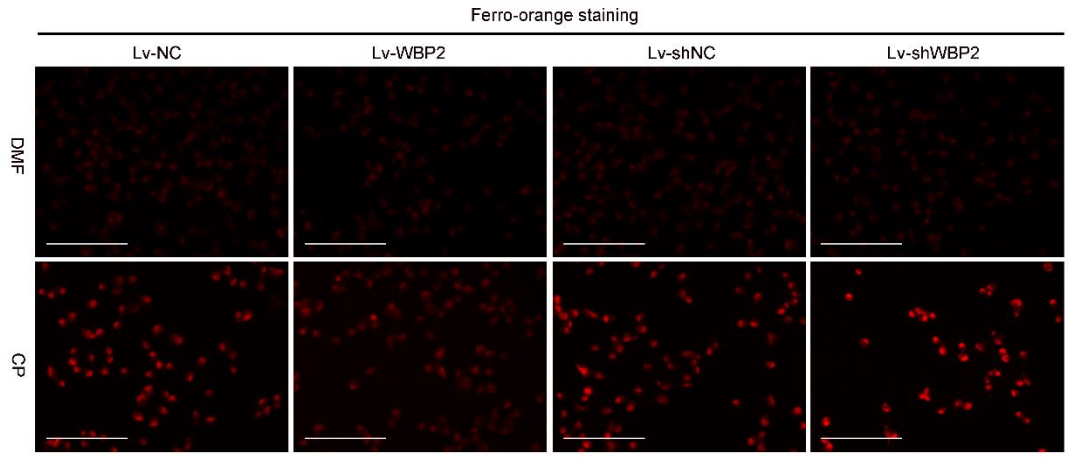
Supplemental Figure 2. WBP2 was downregulated in CP-AKI.

A Immunofluorescence staining showed that WBP2 (green) was colocalized megalin (red), suggesting that it was abundantly expressed in renal proximal tubules; **B-C** Immunohistochemistry staining showed that WBP2 was downregulated in cisplatin-treated kidneys. DMF: dimethylformamide, CP: cisplatin. Scar bars:100 μm.



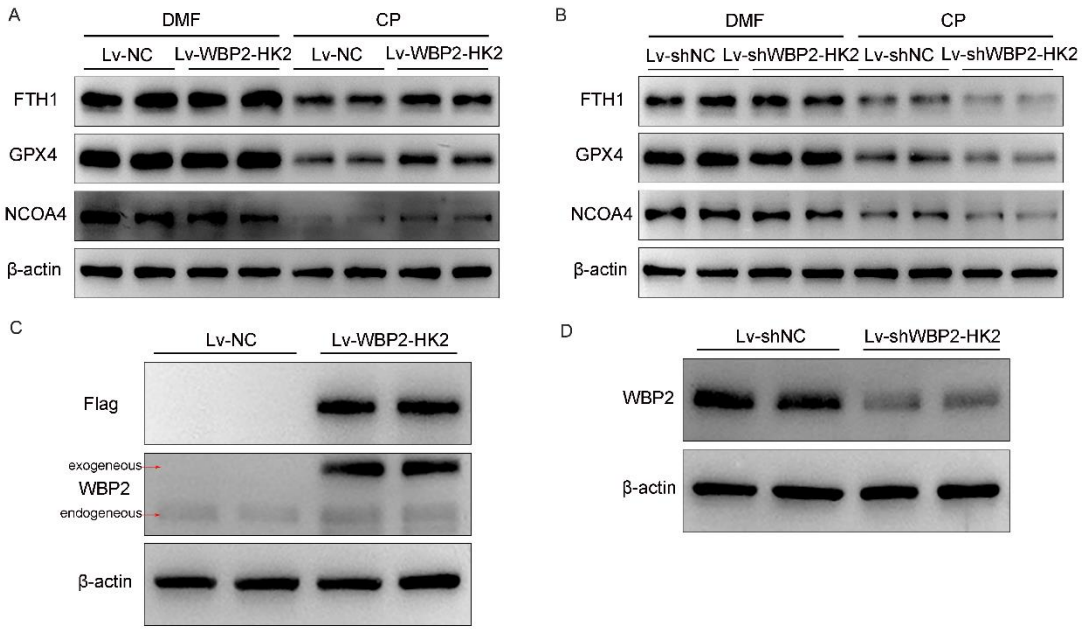
Supplemental Figure 3. GFP staining of renal sections

Immunofluorescence staining showed that GFP was expressed in adenovirus-injected kidneys. Scale bars: 100 μm.



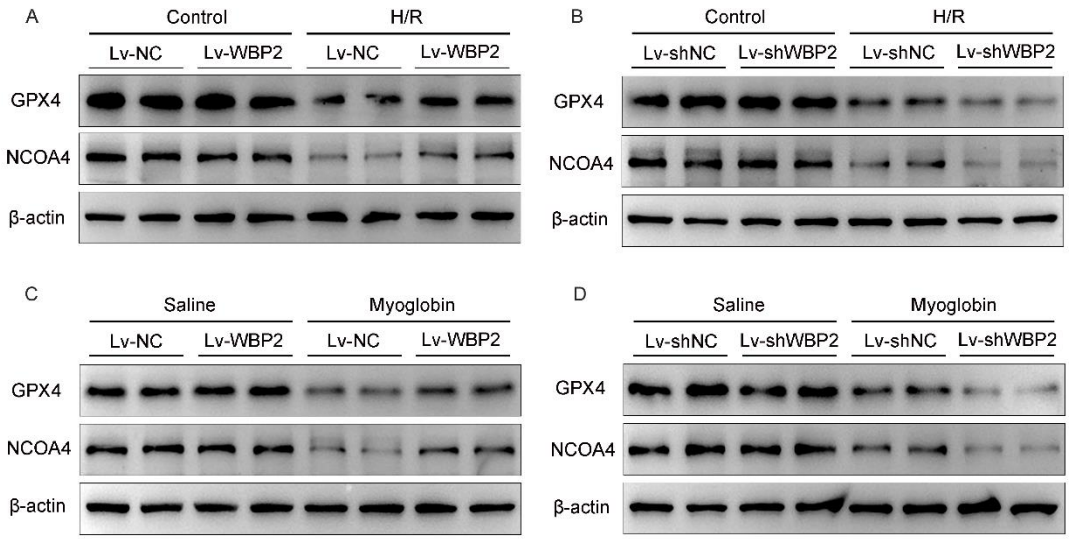
Supplemental Figure 4. FerroOrange staining of BUMPT cells.

Labile iron levels, as evaluated by FerroOrange staining, were increased following cisplatin treatment, which was attenuated by WBP2 overexpression but accentuated by WBP2 knockdown. DMF: dimethylformamide, CP: cisplatin, Lv-NC: empty vector lentivirus for WBP2 overexpression, Lv-WBP2: lentivirus-mediated WBP2 overexpression, Lv-shNC: empty vector lentivirus for WBP2 knockdown, Lv-shWBP2: lentivirus-mediated WBP2 knockdown. Scale bars: 100 μm.



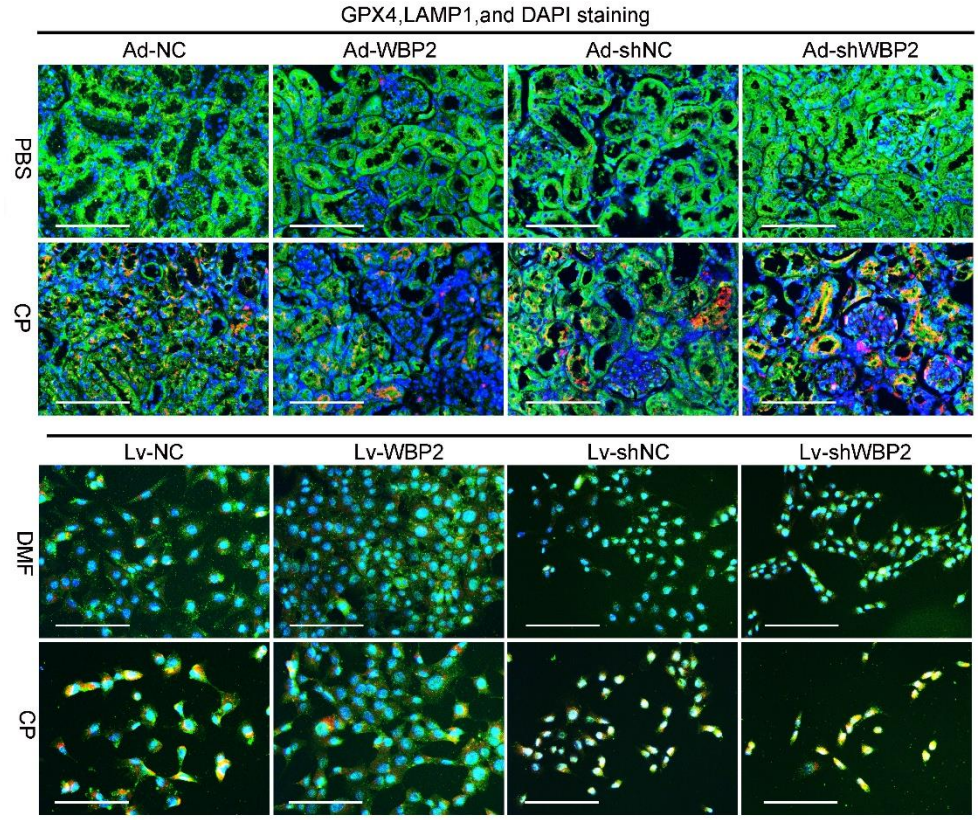
Supplemental Figure 5. WBP2 decelerated ferroptosis to alleviate cisplatin-induced cell death in HK-2 cells.

A - **B** Western blot studies demonstrated that WBP2 overexpression mitigated, while its gene disruption aggravated, cisplatin-induced degradation of FTH1, NCOA4 and GPX4 in HK-2 cells; **C – D** Western blot studies of Flag and WBP2 showed that WBP2 expression was manipulated by lentivirus transfection. DMF: dimethylformamide, CP: cisplatin, Lv-NC: empty vector lentivirus for WBP2 overexpression, Lv-WBP2-HK-2: lentivirus-mediated WBP2 overexpression for HK-2 cells, Lv-shNC: empty vector lentivirus for WBP2 knockdown, Lv-shWBP2-HK-2: lentivirus-mediated WBP2 knockdown for HK-2 cells.



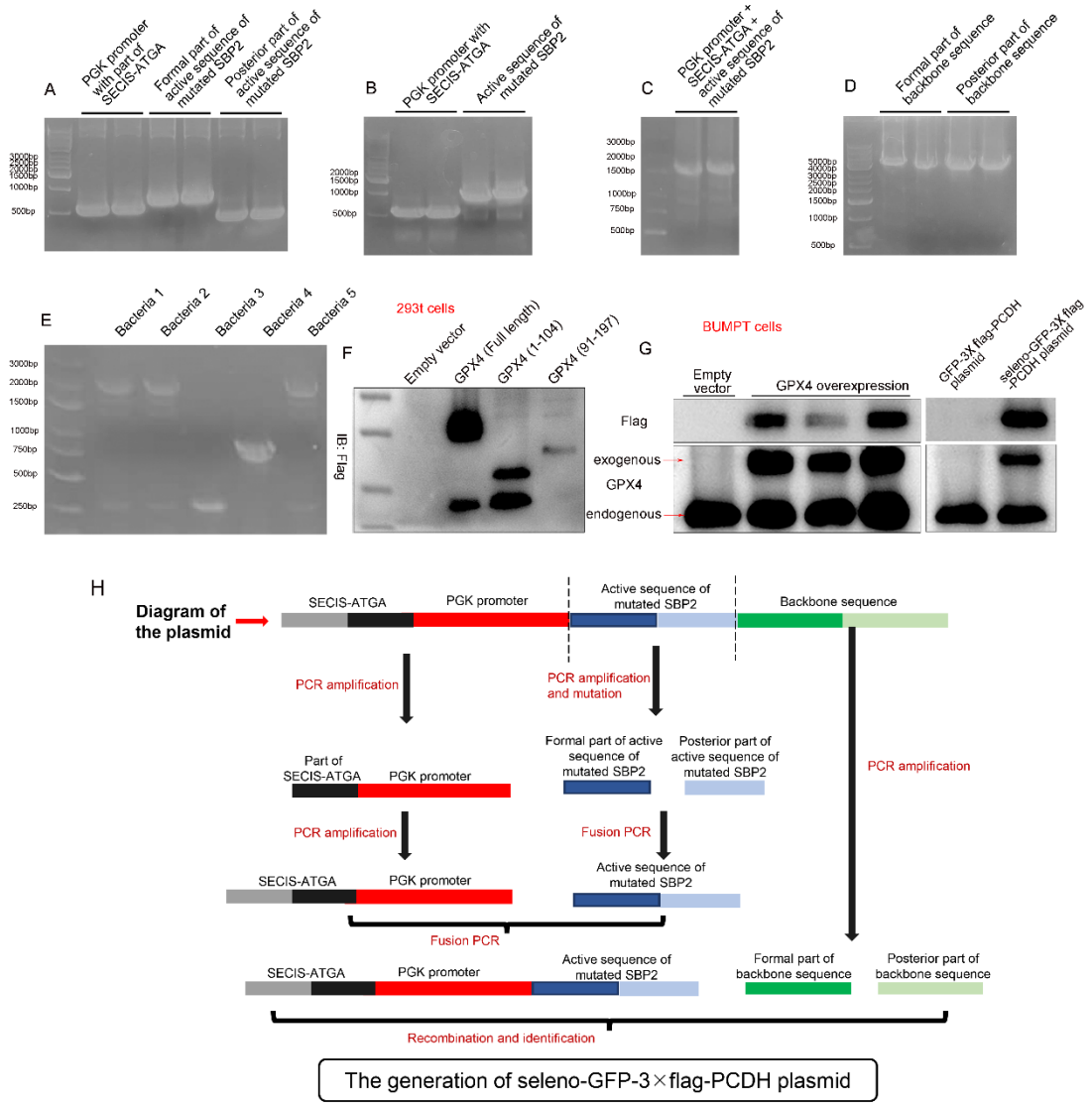
Supplemental Figure 6. WBP2 inhibited ferroptosis to alleviate hypoxia/reoxygenation or myoglobin-induced cell death in BUMPT cells.

A - B Western blot studies demonstrated that WBP2 overexpression mitigated, while its gene disruption aggravated, hypoxia/reoxygenation-induced degradation of NCOA4 and GPX4; **C - D** Western blot studies demonstrated that WBP2 overexpression mitigated, while its gene disruption aggravated, myoglobin-induced degradation of NCOA4 and GPX4. DMF: dimethylformamide, CP: cisplatin, H/R: hypoxia/reoxygenation, Lv-NC: empty vector lentivirus for WBP2 overexpression, Lv-WBP2: lentivirus-mediated WBP2 overexpression, Lv-shNC: empty vector lentivirus for WBP2 knockdown, Lv-shWBP2-HK-2: lentivirus-mediated WBP2 knockdown.



Supplemental Figure 7. CO-immunofluorescence staining of GPX4 and LAMP1.

CO-immunofluorescence staining showed that GPX4 (green) co-localized with lysosomes (red, marked by LAMP1) in cisplatin-treated BUMPT cells and kidneys, and their co-localization (yellow, merged fluorescence) was attenuated by WBP2 overexpression but accentuated by WBP2 knockdown; DMF: dimethylformamide, CP: cisplatin, Lv-NC: empty vector lentivirus for WBP2 overexpression, Lv-WBP2: lentivirus-mediated WBP2 overexpression, Lv-shNC: empty vector lentivirus for WBP2 knockdown, Lv-shWBP2: lentivirus-mediated WBP2 knockdown, Ad-NC: empty vector adenovirus for WBP2 overexpression, Ad-WBP2: adenovirus-mediated WBP2 overexpression, Ad-shNC: empty vector adenovirus for WBP2 knockdown, Ad-shWBP2: adenovirus-mediated WBP2 knockdown. Scale bars: 100 μm.



Supplemental Figure 8. The generation of seleno-GFP-3×flag-PCDH plasmid.

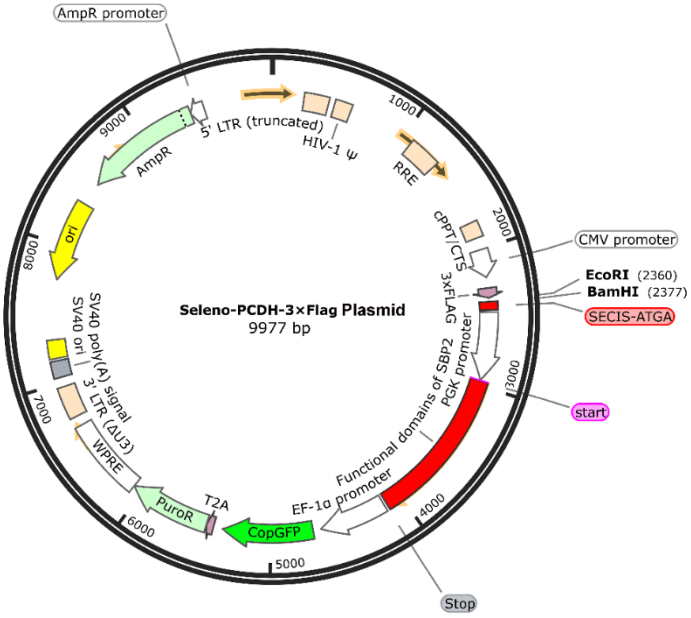
A - E Products of different PCR procedures; **F – G** Western blot studies showed that GPX4 and its truncated forms were expressed by seleno-GFP-3×flag-PCDH plasmid, and GPX4 cannot be expressed by GFP-3×flag-PCDH plasmid; **H** The diagram for the generation of seleno-GFP-3×flag-PCDH plasmid.

Introduction for the generation of seleno-GFP-3×flag-PCDH plasmid.

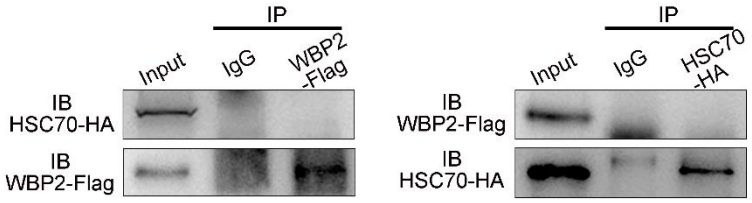
In order to express GPX4 (a unique selenoprotein) in mammal cells, selen-GFP-3×flag-PCDH plasmid (a lentivirus plasmid) was generated with multiple PCR procedures. The synthesis of selenoproteins incorporates a key procedure, where selenocysteine (Sec) is recognized by UGA, a stop codon in normal state(1). This unique type of transcription relies on the Sec insertion sequence (SECIS) element in the 3' untranslated regions and SECIS-binding protein 2 (SBP2)(2). Multiple types of SECIS element have been identified, and previous publication showed that ATGA form of Toxoplasma SelT SECIS (SECIS-ATGA) element conceived the highest activity in Sec insertion(3). The sequence of SECIS-ATGA element is as following: CTGCGAATGAGGATGCTGGCAGAAACCTCTCCATTCGA GGCAGCTGGCATCTGATAGTTGG(3). Besides, in order to reduce the size of the plasmid

to facilitate lentivirus packaging, only a minimal active sequence of SBP2 (mice, 1222-2367bp of its cDNA) was subcloned into the plasmid(2). Noteworthily, null mutations were performed in the active sequence of SBP2 to erase the sites recognized by EcoR I and BamH I, which were also presented in multiple cleavage sits of GFP-3×flag-PCDH plasmid. PGK promoter was used to drive the expression of SBP2 sequence.

The procedures were introduced as follows: Firstly, PGK promoter was initially cloned, and two PCR procedures were used to add SECIS-ATGA element into the 5' terminal of PGK promoter sequence (Supplemental Figure 3A, lanes 1 & 2, and Supplemental Figure 3B, lanes 1 & 2). Secondly, the active sequence of SBP2 was null mutated with pre-designed primers, which divided the sequence into two parts (Supplemental Figure 3A, lanes 3 - 6). Fusion PCR procedure was used to obtain the full length of the mutated active sequence of SBP2 (Supplemental Figure 3B, lanes 3 & 4). Thirdly, another fusion PCR procedure was employed to link the mutated active sequence of SBP2 and the sequence of SECIS-ATGA element and PGK promoter (Supplemental Figure 3C). Fourthly, the backbone sequence of GFP-3×flag-PCDH plasmid was cloned. Noteworthily, the backbone sequence was divided into two parts to decrease the rate of mutation during PCR amplification (Supplemental Figure 3D). The PCR products of the third step and the fourth step were purified and recombined, and the mixtures were transformed into DH5α cells. Fifthly, PCR-mediated bacteria identification was used, and three bacterial colonies with positive results were picked up and amplified (Supplemental Figure 3E). The successful generation of seleno-GFP-3×flag-PCDH plasmid was further confirmed by plasmid sequencing. Finally, seleno-GFP-3×flag-PCDH plasmid was cleaved by EcoR I and BamH I, and GPX4 and its truncated sequences were subcloned into the plasmid. The expression of GPX4 and its truncated forms were expressed in seleno-GFP-3×flag-PCDH plasmid, while full-length GPX4 cannot be expressed in GFP-3×flag-PCDH plasmid, as validated by western blot studies (Supplemental Figure 3F-3G). The procedures were briefly summarized in Supplemental Figure 3H.



Supplemental Figure 9. The gene map of seleno-GFP-3×flag-PCDH plasmid.



Supplemental Figure 10. WBP2 cannot bind with HSC70 in BUMPT cells.

CO-IP studies showed that WBP2 (Flag-tag) cannot co-precipitate with HSC70 (HA-tag) in BUMPT cells.

Supplemental Table 1. The primers used in our present studies.

Dataset	Sample type	PMID	Country	Platform	Samples (AKI)	Samples (control)
GSE1563	kidney	15307835	USA	Affy	28	11
GSE30718	kidney	22343120	Canada	Affy	7	9
GSE61739	kidney	25808278	USA	Affy	48	48

Supplemental Table 2. List of transcriptome datasets of AKI patients.

Supplemental Table 3. The Number of genes overexpressed or underexpressed in patients with AKI.

Supplemental Table 4. Ferroptosis-related gene network in the context of kidney tissue.

Supplemental Table 5. Scores of hub genes based on 12 algorithms in CytoHubba.

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1. Turanov AA, Lobanov AV, Hatfield DL, and Gladyshev VN. UGA codon position-dependent incorporation of selenocysteine into mammalian selenoproteins. *Nucleic Acids Res.* 2013;41(14):6952-9.
2. Donovan J, and Copeland PR. Threading the needle: getting selenocysteine into proteins. *Antioxid Redox Signal.* 2010;12(7):881-92.

3. Novoselov SV, Lobanov AV, Hua D, Kasaikina MV, Hatfield DL, and Gladyshev VN. A highly efficient form of the selenocysteine insertion sequence element in protozoan parasites and its use in mammalian cells. *Proc Natl Acad Sci U S A.* 2007;104(19):7857-62.