Supplementary Methods

Cell viability assay

The cell counting kit-8 (CCK-8) assay was conducted to detect the viability of HK-2 cells. Briefly, cells were seeded in 96-well plates at 6,000 cells/well, then, the plates were incubated at 37 °C and 5% CO_2. After the cells adhered, they were treated with cisplatin for the scheduled time. Then, removing the medium before adding the 200-μL CCK-8 solution (Dojindo Laboratories) and incubating at 37 °C for 2 hours, the absorbance value of each well was measured at 450 nm using a microplate reader.

Immunohistochemistry

Immunohistochemistry was performed following the instructions of the SABC anti-rabbit-POD kit (SA1028; Boster Bioengineering). Briefly, paraffin sections were dewaxed and rehydrated, and antigenically repaired in citrate buffer. Sections were treated with 3% H_2O_2 to block endogenous peroxidase activity and subsequently blocked with 5% goat serum. Afterward, the sections were incubated with specific primary antibodies at 4 °C overnight. After being washed with phosphate-buffered saline, the sections were incubated with horseradish peroxidase (HRP)-labeled secondary antibody at room temperature for 1 hour. DAB was used for chromatography, and then, the sections were restained with hematoxylin, and finally blocked with neutral gum after dehydration and hyaluronidation. The staining results were observed under a light microscope and images were captured and analyzed. The antibodies used were as following: methylcrotonyl-CoA carboxylase 2 (MCCC2) (Abclonal; A15181, 1:100), secondary antibody anti-Rabbit (Abclonal; AS014, 1:100).

Western blot

The kidney tissues were removed and weighed, and then lysed with radio-immunoprecipitation assay buffer (Cwbio) containing protease inhibitors (Cwbio) for 30 minutes on ice. Mitochondrial protein was extracted using a mitochondrial protein extraction kit (Beyotime Biotechnology). The protein concentration of the sample was measured by a bicinchoninic acid protein assay kit (Biosharp) and denatured by incubation at 100 °C for 10 minutes. An equivalent quantity of protein was separated on a 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis gel (Bio-Rad) and transferred to polyvinylidene fluoride (PVDF) membranes (Millipore). PVDF membranes were placed in 5% skim milk diluted with Tris-buffered saline with Tween 20 buffer and incubated for 1 hour and then placed in primary antibody and incubated overnight at 4 °C. After incubation, HRP-conjugated secondary antibody was used at room temperature for 1 hour. Proteins were visualized using enhanced chemiluminescence (Millipore) and quantified using ImageJ software. The antibodies used for this experiment were as follows: MCCC2 (Proteintech; 12117-1-AP, 1:1,000), COX IV (Proteintech; 66110-1-lg, 1:10,000), β-actin (Abclonal; AC026, 1:20,000), secondary antibody anti-Mouse (Abclonal; AS003, 1:10,000), secondary antibody anti-Rabbit (Abclonal; AS014, 1:10,000).

Immunofluorescence

Tissue slides were dewaxed, rehydrated, and subjected to antigen retrieval, then blocked for 1 hour. The sections were then incubated with primary antibody dilution overnight at 4 °C. After three times washing, secondary antibody dilutions were used to incubate the sections for 1 hour at room temperature. To see the nuclei, cells were then labeled with DAPI (4',6-diamidino-2-phenylindole) for 10 minutes at room temperature. Finally, the sections were analyzed by an integrated fluorescence microscope imaging system (Keyence) and fluorescence intensity analysis was performed by ImageJ software. The antibodies used were as following: MCCC2 (Abclonal; A15181, 1:100), cytochrome c oxidase IV (COX IV) (Proteintech; 66110-1-lg, 1:100), 594-conjugated Goat anti-Mouse (Proteintech; SA00013-3, 1:250), 488-conjugated Goat anti-Rabbit (Abclonal; AS053, 1:250).