Supplementary Figure 1: The alterations in ferroptosis markers, induced by Cisplatin treatment in CD1 mice, are alleviated by the administration of Fer-1. Immuno-blotting studies revealed that the expression levels of ferroptosis markers, including 4-HNE, NCOA4 and FTH1, underwent substantial changes following Cisplatin treatment in CD1 mice. Interestingly, these alterations were attenuated by the pre-treatment of Fer-1.

Supplementary Figure 2: Cisplatin-induced tubular injury was alleviated by Fer-1 treatment two hours prior to the induction of AKI. Cisplatin treatment lead to severe tubular damage, which was attenuated by the administration of Fer-1 (two hours before the treatment of Cisplatin), as indicated by H & E staining (panels A - C). Similarly, Cisplatin-treated mice had a considerable increase in the serum creatinine levels and the mRNA levels of KIM-1 and NGAL, which was attenuated by two hours’ prior treatment of Fer-1 (panels D - F) (n=4; * p < 0.05 compared with the WT control group, # p < 0.05 compared with the WT CP group, 1-way ANOVA with Dunn's multiple comparisons). Scale bar: 30 μm.
Supplementary Figure 3: Cisplatin treatment (four hours) leads to up-regulation of MIXO and depletion of FTH in HK-2 cells, which can be attenuated by the co-treatment of Mito Q. MIOX expression levels were upregulated while FTH1 was degradated after 4 hours’ cisplatin treatment in HK-2 cells. Those expression changes of MIOX and FTH1 was partially restored by Mito Q treatment.

Supplementary Figure 4: Cisplatin-induced FTH1 degradation was attenuated by the administration of NCOA4 SiRNA in MIOX overexpressing HK-2 cells. Immuno-blotting studies revealed that FTH1 expression levels decreased in MIOX-overexpressing HK-2 cells following Cisplatin treatment, which was alleviated by the transfection of NCOA4 SiRNA.
Supplementary Figure 5: Cisplatin-induced tubular damage in MIOX-TG mice was alleviated by Fer-1 treatment. HE staining showed that the severe tubular damage, induced by Cisplatin treatment in MIOX-TG mice, was considerably attenuated by Fer-1 treatment (panels A - C). The elevated serum creatinine levels in Cisplatin-treated MIOX-TG mice were also alleviated by Fer-1 treatment (panels D) (n=6; * p < 0.05 compared with the WT control group, # p < 0.05 compared with the WT CP group, 1-way ANOVA with Dunn’s multiple comparisons). Besides, Fer-1 treatment reduced the increased mRNA levels of NGAL and KIM-1 in Cisplatin-treated MIOX-TG mice (panels E & F) (n=4; * p < 0.05 compared with the WT control group, # p < 0.05 compared with the WT CP group, 1-way ANOVA with Dunn’s multiple comparisons). Scale bar: 30 μm.

Supplementary Figure 6: Cisplatin treatment (2 hours) leads to the downregulation of NCOA4 and upregulation of FTH1 in mice. Immuno-blotting studies showed that NCOA4 was down regulated, while FTH1 was upregulated, in the kidneys two hours after the Cisplatin treatment.