

Fig.S1 Tim-AIII inhibits cell proliferation and induces cell death in LLC and SPC-A1 cells. **A** The LLC and SPC-A1 cells viability was measured after treatment with different concentration of Tim-AIII in different time periods. **B** The OD value of LDH in the supernatant of LLC and SPC-A1 cells after treatment with different concentration of Tim-AIII in different time periods. Quantitative data were presented as mean \pm SD. * p < 0.05, ** p < 0.01, *** p < 0.001.

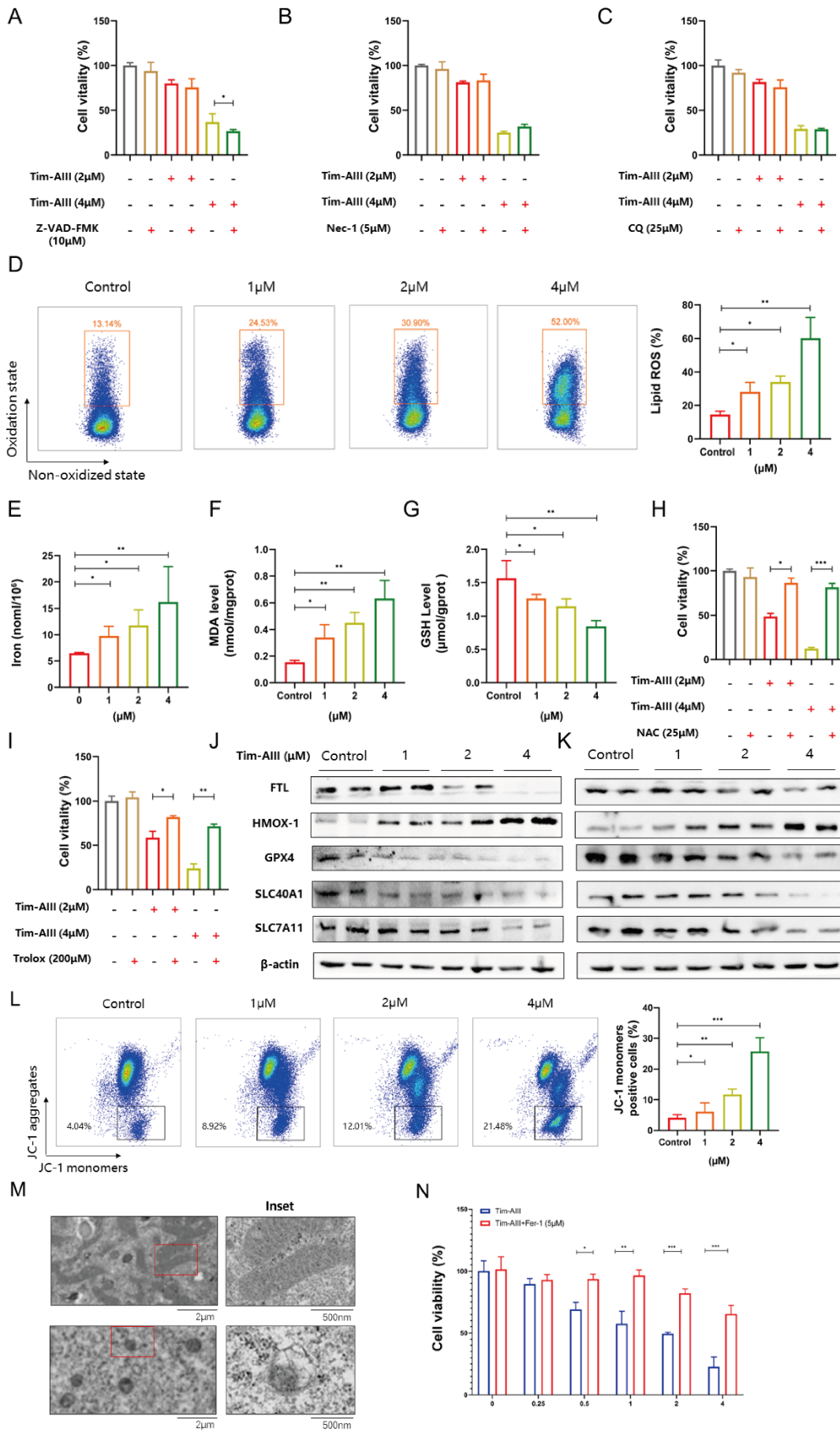


Fig. S2 Tim-AIII-induced cell death is mainly caused by ferroptosis in A549 cells. **A-C** A549 cells were cotreated with Tim-AIII with or without Z-VAD-FMK, Nec-1 and CQ for 48h, and the cell vitality was assayed by CCK-8 assay. **D** The lipid ROS level was analyzed by a flow cytometer after treatment with Tim-AIII for 48h in A549 cells. **E** The intracellular iron level after treatment with Tim-AIII for 48h in A549 cells. **F** The intracellular MDA level after treatment with Tim-AIII for 48h in A549 cells. **G** The intracellular GSH level after treatment with Tim-AIII for 48h in A549 cells. **H-I** A549 cells were cotreated with Tim-AIII with or without the ROS inhibitor NAC and Trolox for 48h, and cell viability was assayed by CCK-8. **J** The expression of several key ferroptosis regulators, such as FLT, HMOX-1, GPX4, SLC40A1, SLC7a11, were examined by western blot in A549 cells. **K** The expression of several key ferroptosis regulators, such as FLT, HMOX-1, GPX4, SLC40A1, SLC7a11, were examined by western blot in LLC cells. **L** Representative flow cytometry results and quantification of mitochondrial membrane potential (JC-1 green) after treatment with Tim-AIII for 48h in A549 cells. **M** TME was used to observe mitochondrion in A549 cells. **N** A549 cells were cotreated with Tim-AIII with or without Fer-1 for 48h, and the cell vitality was assayed by CCK-8 assay. Quantitative data were presented as mean \pm SD. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

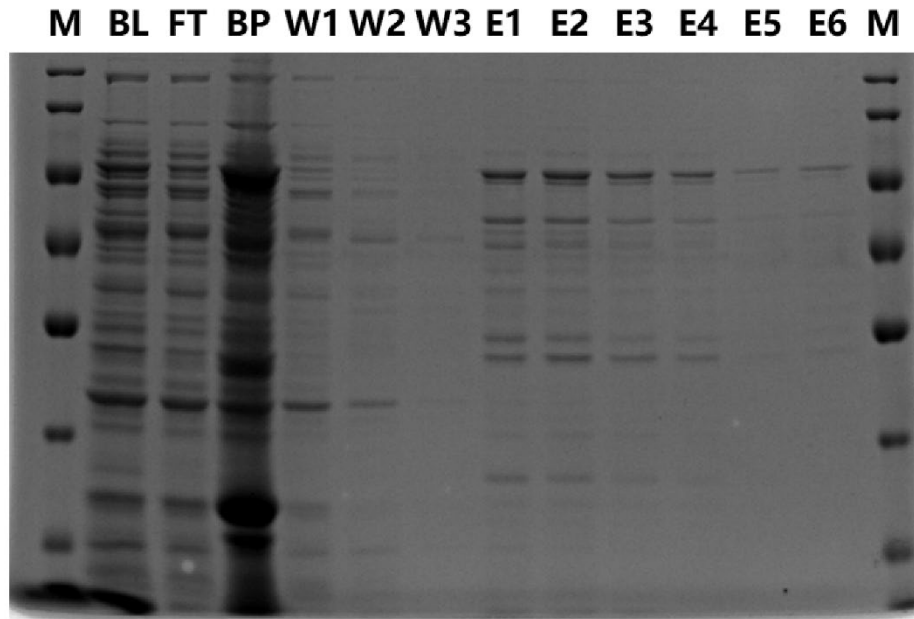


Fig. S3 Coomassie brilliant blue staining effect of purified recombinant HSP90 mutant protein with His tag. M: Marker; BL: bacterial lysate; FT: sample filling fluid; BP: bacterial precipitation; W1-W3: cracking liquid washing 1-3 (Wash 1-3); E1-E6: The eluate contains purified protein.

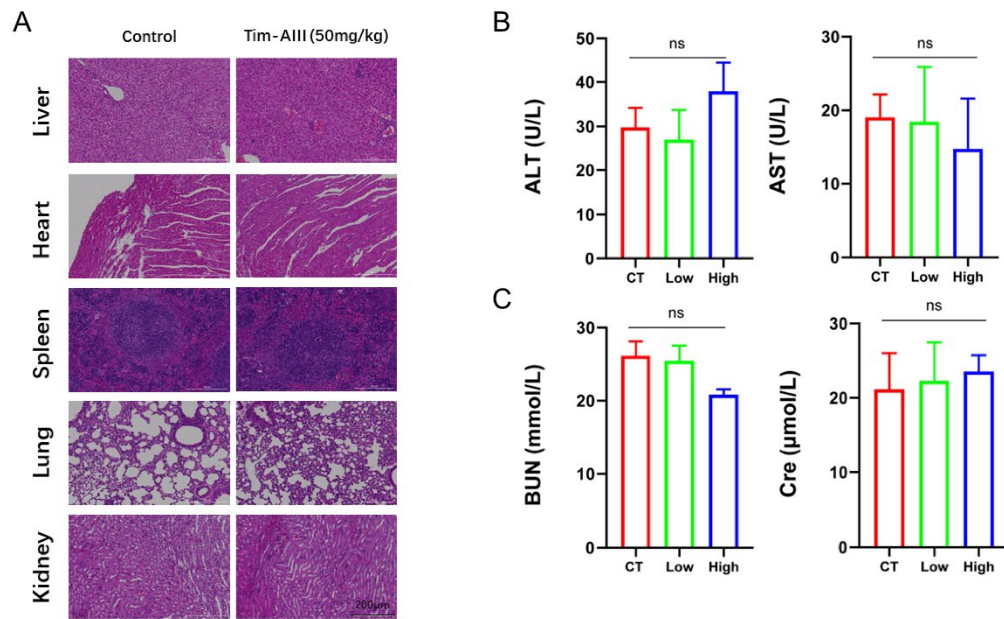


Fig. S4 Tim-AIII is non-toxic to the mice. **A** Hematoxylin and eosin (H&E) staining of liver, heart, spleen, lung and kidney collected from the mice of the high dose treatment and the PBS groups. **B** Serum ALT and AST levels in Tim-AIII administration groups compared with the control group. **C** Serum BUN and Cre levels in Tim-AIII administration groups compared with the control group. Quantitative data were presented as mean \pm SD. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.