Figure S1. Ferroptosis induced by cisplatin is suppressed in cisplatin-resistant A2780 cells (A2780cis cells). (A) Cell viability of A2780 cells treated with (+) or without (-) cisplatin in the absence or presence of deferoxamine for 48 h was measured by WST8 assay. Data are expressed as the mean ± SD (n=5; *P<0.05 and ***P<0.001; Tukey's honestly significant difference test). (B) The mean fluorescence intensities of Liperfluo in A2780 and A2780cis cells treated with (+) or without (-) cisplatin for 48 h were measured by flow cytometric analysis. Relative mean fluorescence intensities were determined by defining that of A2780 cells without cisplatin treatment as 1. Data are expressed as the mean ± SD (n=3; ***P<0.001; Tukey's HSD test).

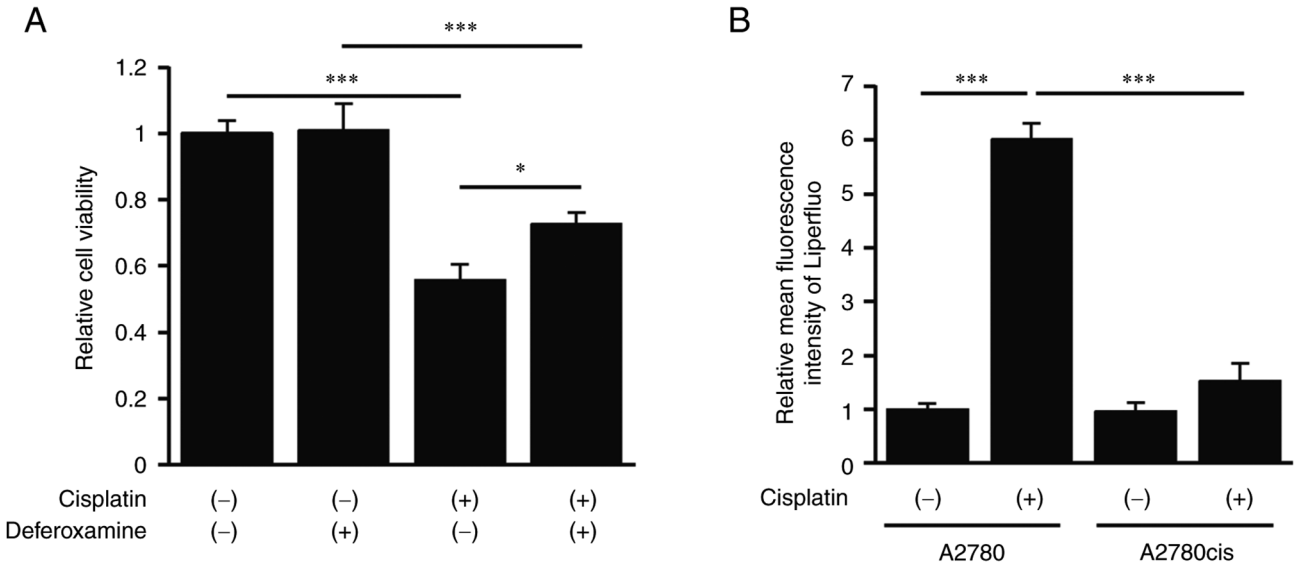

Figure S2. Copper ions are not involved in cisplatin-mediated cell death. (A and B) Relative activities of SOD in (A) A2780 cellsand (B) OVK18 cells treated with (+) or without (-) d-penicillamine were analyzed by SOD assay. Data are expressed as the mean ± SD (n=3) (*P<0.05, Student's t-test). (C and D) Cell viability of (C) A2780 cells and (D) OVK18 cells treated with (+) or without (-) cisplatin in the absence or presence of d-penicillamine was analyzed by WST8 assay. Data are expressed as the mean ± SD (n=3; *P<0.05 and **P<0.01; Tukey's honestly significant difference test). SOD, superoxide dismutase; n.s., not significant.

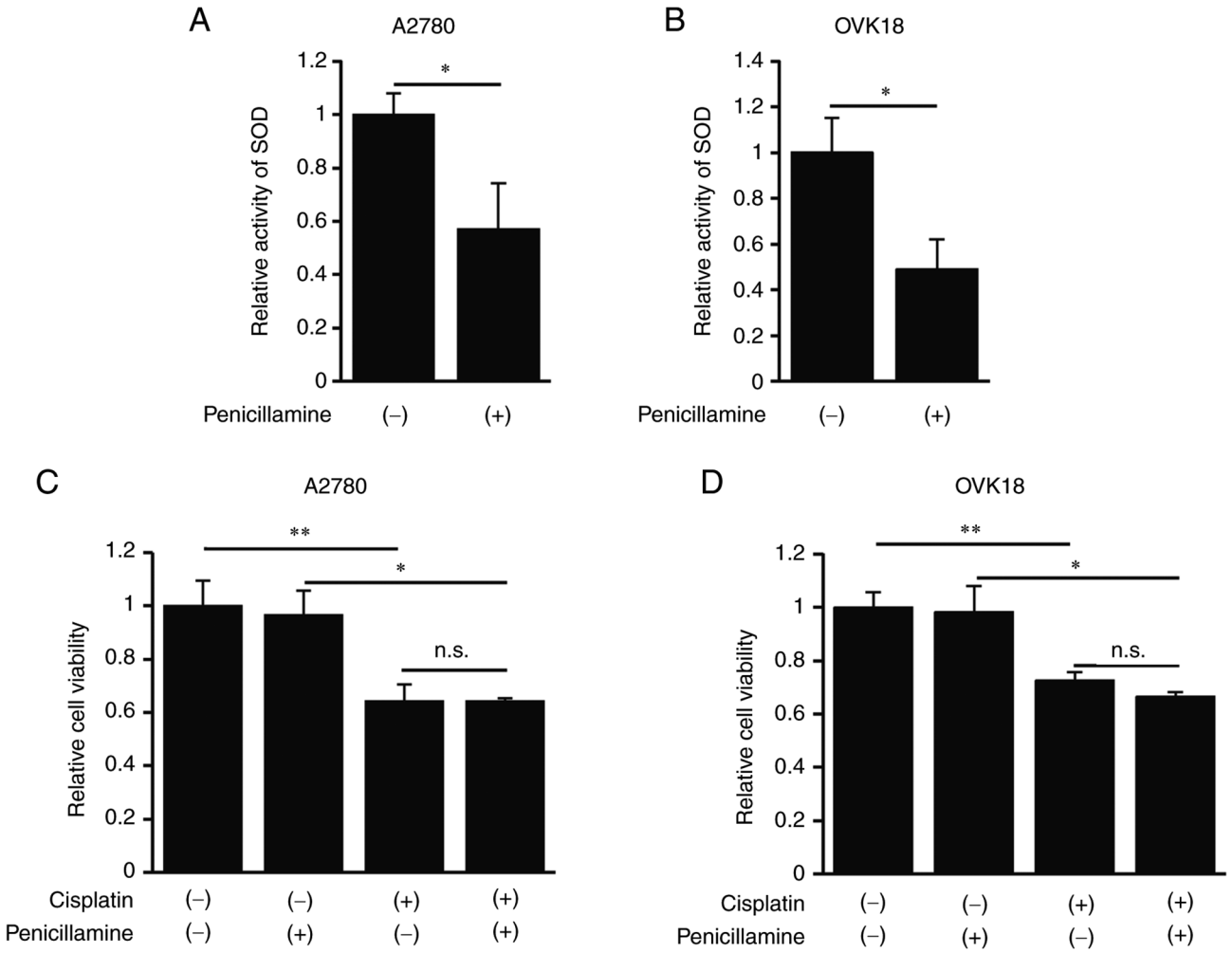

Figure S3. Fdx1 inhibits cisplatin-induced cell death in cisplatin-resistant A2780 cells (A2780cis cells). (A) Expression of Fdx1 and α-tubulin in A2780 and A2780cis cells transfected with the indicated siRNAs was evaluated by western blot analysis. Relative band intensities of Fdx1 normalized by that of α-tubulin were determined (right graph). Data are expressed as the mean ± SD (n=3; **P<0.01 and ***P<0.001; Tukey's HSD test). (B) Cell viability of A2780cis cells transfected with either control or Fdx1 siRNAs (#1, #2) followed by treatment with cisplatin for 48 h was measured by WST8 assay. Data are expressed as the mean ± SD (n=3; **P<0.01 and ***P<0.001; Tukey's HSD test). Fdx1, ferredoxin1; siRNA, small interfering RNA; HSD, honestly significant difference.

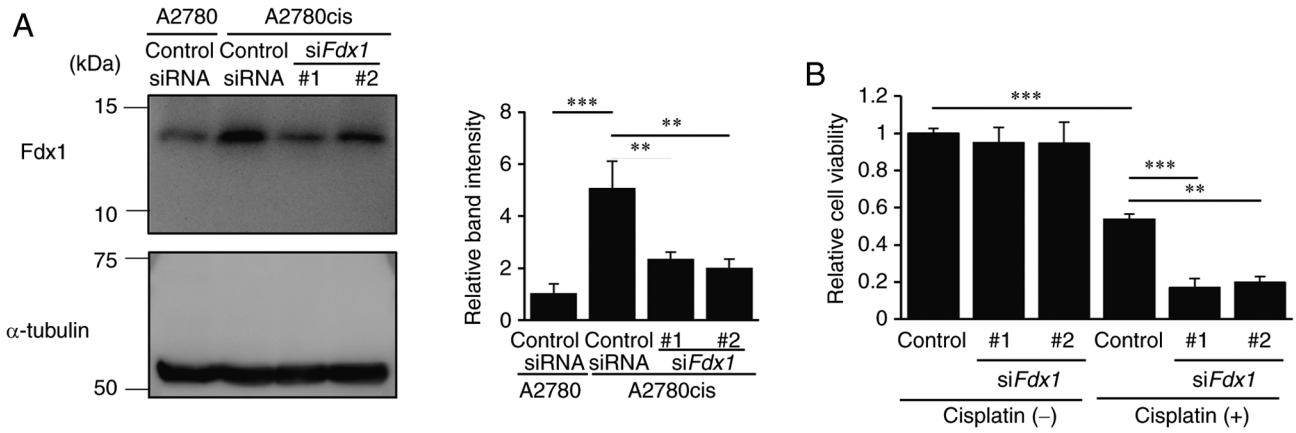

Figure S4. Cisplatin-induced ferroptosis via upregulated mitochondrial membrane potential and upregulated lipid peroxidation is suppressed in cisplatin-resistant A2780 cells (A2780cis cells). (A) The mean fluorescence intensities of MT1 in A2780 cells treated with or without rotenone for 48 h in the presence of cisplatin were measured by flow cytometric analysis. Relative mean fluorescence intensities were determined by defining that of A2780 cells without rotenone as 1. Data are expressed as the mean ± SD (n=5; *P<0.05; Student's t-test). (B) The mean fluorescence intensities of Liperfluo in A2780 cells treated with or without rotenone for 48 h in the presence of cisplatin were measured by flow cytometric analysis. Relative mean fluorescence intensities were determined by defining that of A2780 cells without rotenone as 1. Data are expressed as the mean ± SD (n=3; *P<0.05; Student's t-test). (C) The mean fluorescence intensities of MT1 in A2780 and A2780cis cells treated with (+) or without (-) cisplatin were measured by flow cytometric analysis. Relative mean fluorescence intensities were determined by defining that of A2780 cells without cisplatin treatment as 1. Data are expressed as the mean ± SD (n=3; ***P<0.001; Tukey's honestly significant difference test).