Figure S1. Viability of PC12 cells treated with different concentrations of FAC for different durations. n=6. *P<0.05, **P<0.01 and ***P<0.001 vs. Control. FAC, ferric ammonium citrate.

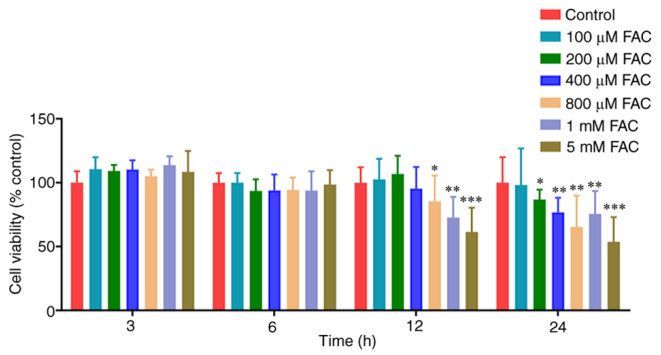

Figure S2. PC12 cells exposed to 0, 200 and 400 μM FAC for 24 h. Level of (A) MDA and (B) SOD activity in PC12 cells. (C) Level of ROS assessed using 2',7'-dichlorodihydrofluorescein diacetate via flow cytometry. (D) Mean fluorescence intensity of ROS. n=3. *P<0.05; **P<0.01; ***P<0.001. FAC, ferric ammonium citrate; MDA, malondialdehyde; SOD, superoxide dismutase; ROS, reactive oxygen species.

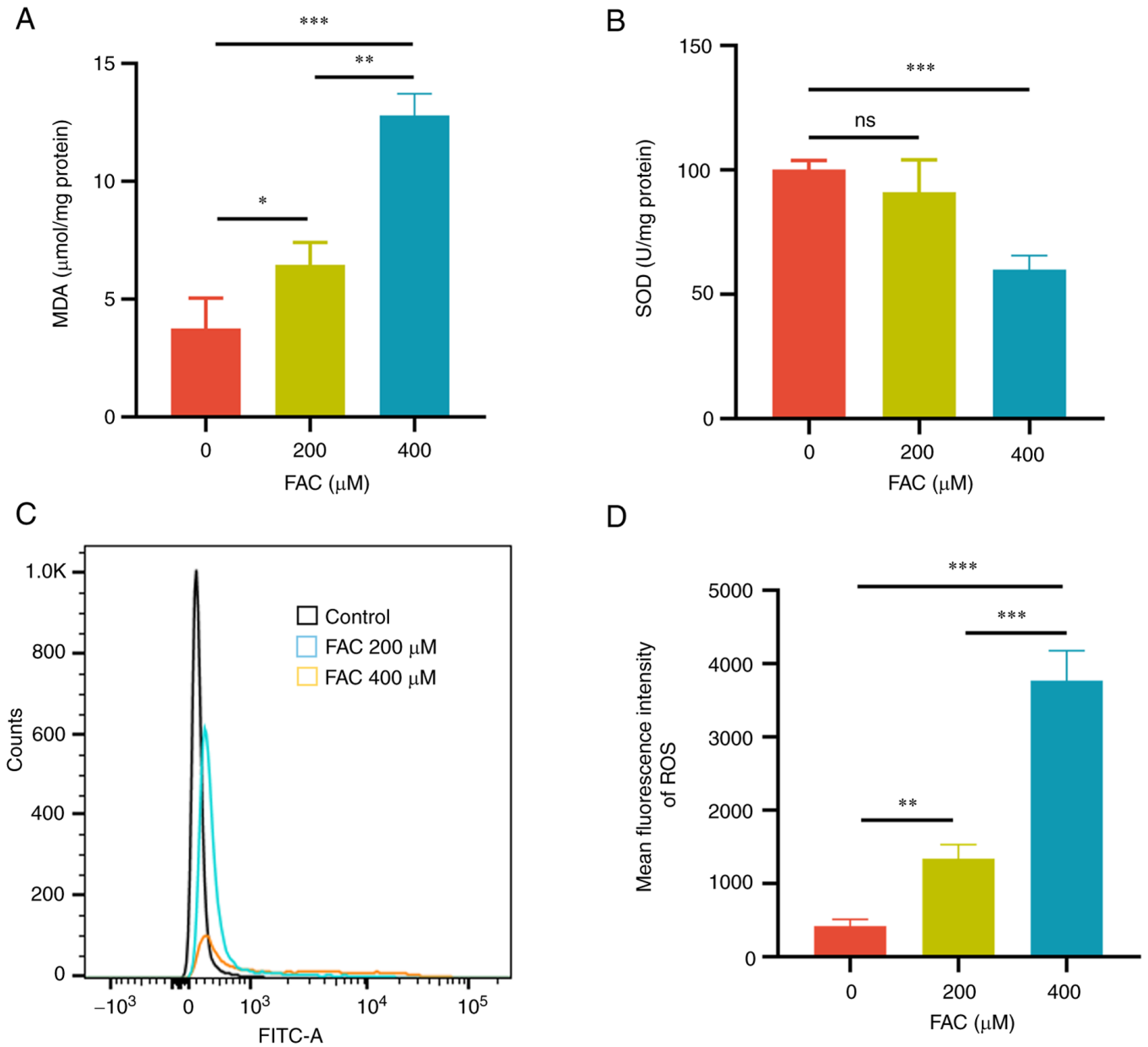

Figure S3. Hemolytic hyperbilirubinemia model was successfully constructed in neonatal Sprague-Dawley rats via PHZ injections. (A) Schematic diagram of the establishment of the hemolytic hyperbilirubinemia model. (B) Changes in appearance of the newborn rats following PHZ injection. (C) Color changes of the brain tissues in the neonatal rats following PHZ injection. Following PHZ injection, the serum levels of (D) hematocrit (E) hemoglobin, (F) total bilirubin, (G) direct bilirubin and (H) indirect bilirubin were evaluated. n=6. ***P<0.001. PHZ, phenylhydrazine.

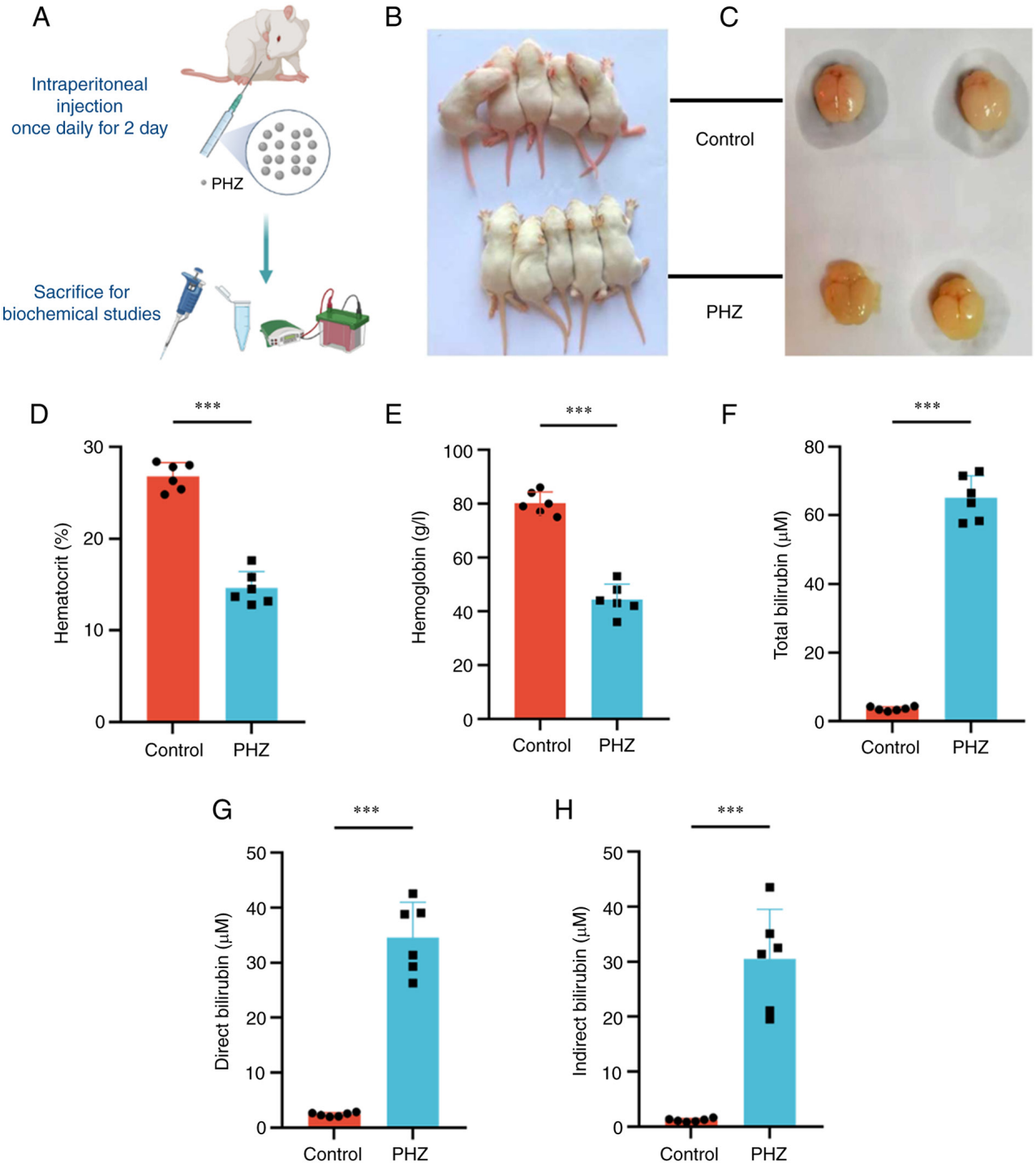

Figure S4. Levels of ferroptosis-associated proteins in the brain of neonatal Sprague-Dawley rats with PHZ-induced hemolytic hyperbilirubinemia. (A) Expression levels of FPN1, xCT and GPX4 proteins, determined using western blotting. Semi-quantitative analysis of the protein expression of (B) FPN1, (C) GPX4 and (D) xCT. n=6. *P<0.05. FPN1, ferroportin 1; GPX4, glutathione peroxidase 4; PHZ, phenylhydrazine; ns, not significant.

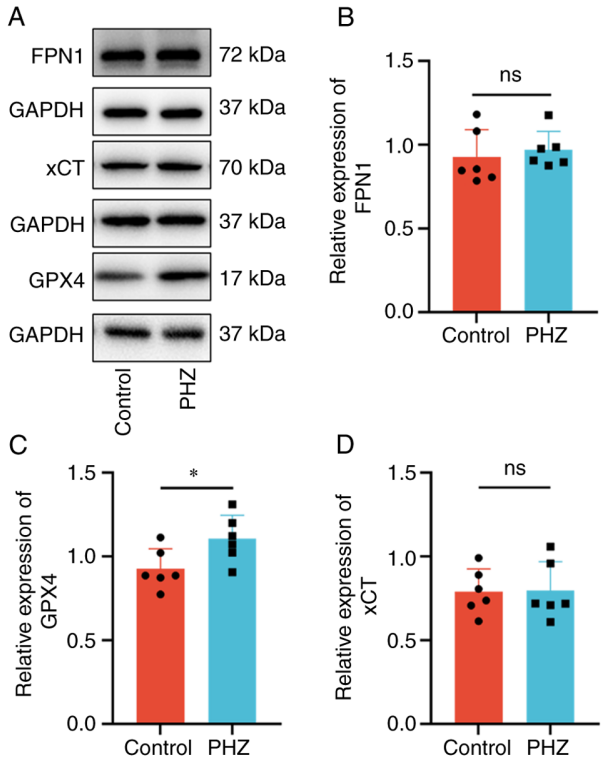

Figure S5. Hemolysis-related indexes are not restored by pretreatment with DFO in the hemolytic hyperbilirubinemia model. Serum levels of (A) hematocrit, (B) hemoglobin, (C) total bilirubin, (D) direct bilirubin and (E) indirect bilirubin. n=6. ***P<0.001. DFO, deferoxamine; PHZ, phenylhydrazine.

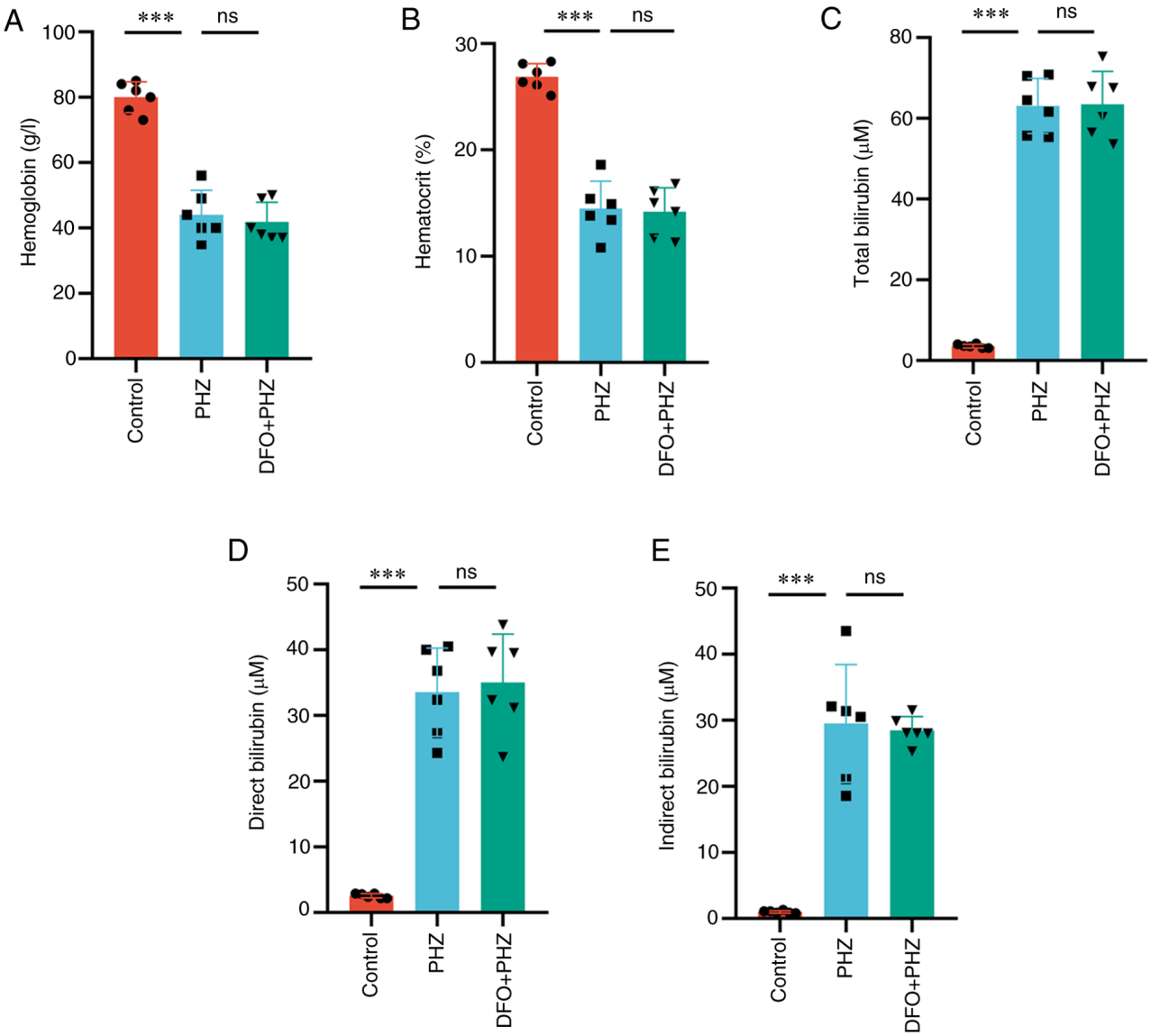

Figure S6. Levels of ferroptosis-associated proteins in the brains of rats with PHZ-induced hemolytic hyperbilirubinemia and DFO pretreatment. (A) Expression levels of FPN1, xCT and GPX4 proteins demonstrated using western blotting. Semi-quantitative analysis of the protein expression of (B) FPN1, (C) GPX4 and (D) xCT. n=6. *P<0.05. PHZ, phenylhydrazine; DFO, deferoxamine; FPN1, ferroportin 1; GPX4, glutathione peroxidase 4; ns, not significant.