

Figure S1. Erastin and RSL3 promote the expression of IRP1 and IRP2 in G-361 melanoma cells. (A and B) mRNA levels of IRP1 and IRP2 were increased in (A) erastin- or (B) RSL3-treated G-361 cells. (C and D) Protein levels of IRP1 and IRP2 were increased in (A) erastin- or (B) RSL3-treated G-361 cells. Data are presented as the mean \pm SD of three independent experiments. * P <0.05; ** P <0.01; *** P <0.001; **** P <0.0001. IRP, iron regulatory protein.

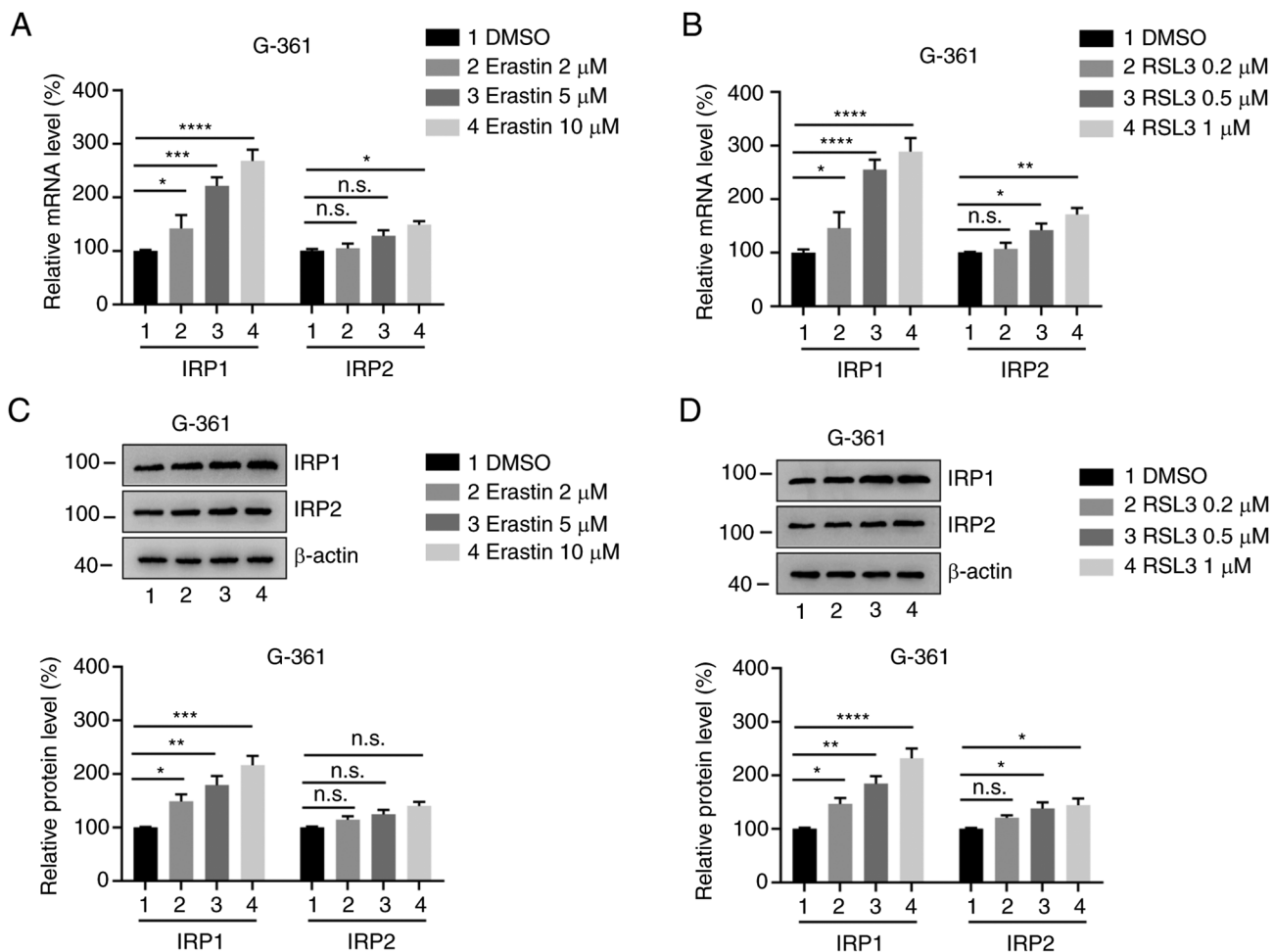


Figure S2. Knockdown of IRP1 and IRP2 inhibits erastin- and RSL3-induced ferroptosis. (A) IRP1 and IRP2 knockdown inhibited erastin- and RSL3-induced ferroptotic cell death in G-361 melanoma cells. (B) Knockdown of IRP1 and IRP2 inhibited erastin- and RSL3-induced iron accumulation in G-361 cells. (C and D) Knockdown of IRP1 and IRP2 suppressed erastin- and RSL3-induced (C) MDA and (D) lipid ROS accumulation. (E) Protein levels of IRP1 and IRP2 were detected following IRP1 and IRP2 knockdown. Data are presented as the mean \pm SD of three independent experiments. * P <0.05; ** P <0.01; *** P <0.001; **** P <0.0001. IRP, iron regulatory protein; IRP, iron regulatory protein; sh, short hairpin RNA.

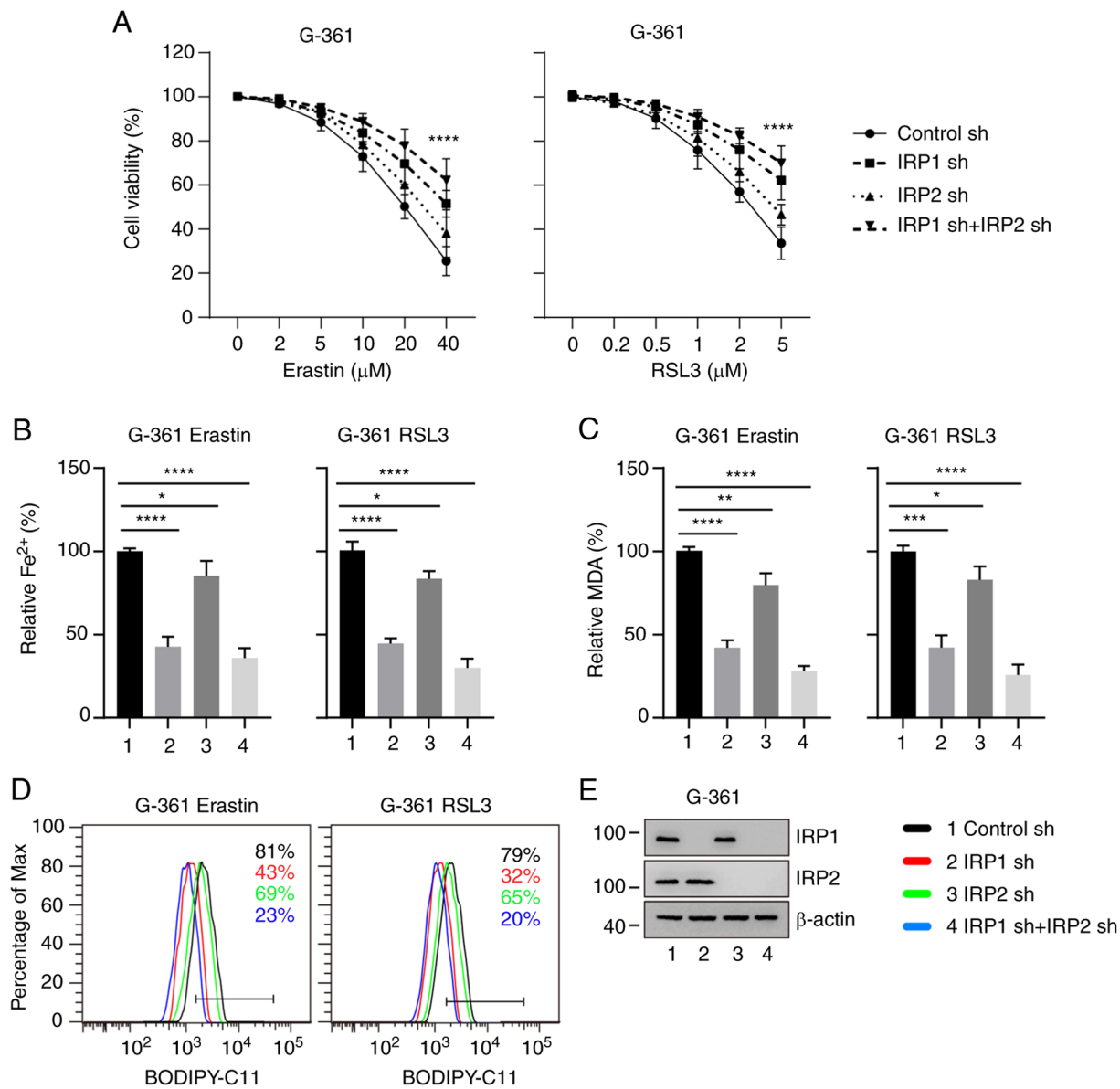


Figure S3. Overexpression of IRP1 and IRP2 promotes erastin- and RSL3-induced ferroptosis. (A) IRP1 and IRP2 overexpression promoted erastin- and RSL3-induced ferroptotic cell death in G-361 melanoma cells. (B) Overexpression of IRP1 and IRP2 increased erastin- and RSL3-induced iron accumulation in G-361 cells. (C and D) Overexpression of IRP1 and IRP2 promoted erastin- and RSL3-induced (C) MDA and (D) lipid ROS accumulation. (E) Protein levels of IRP1 and IRP2 were detected following IRP1 and IRP2 overexpression. Data are presented as the mean \pm SD of three independent experiments. * P <0.05; ** P <0.01; *** P <0.001; **** P <0.0001. IRP, iron regulatory protein; MDA, malondialdehyde; HA, hemagglutinin.

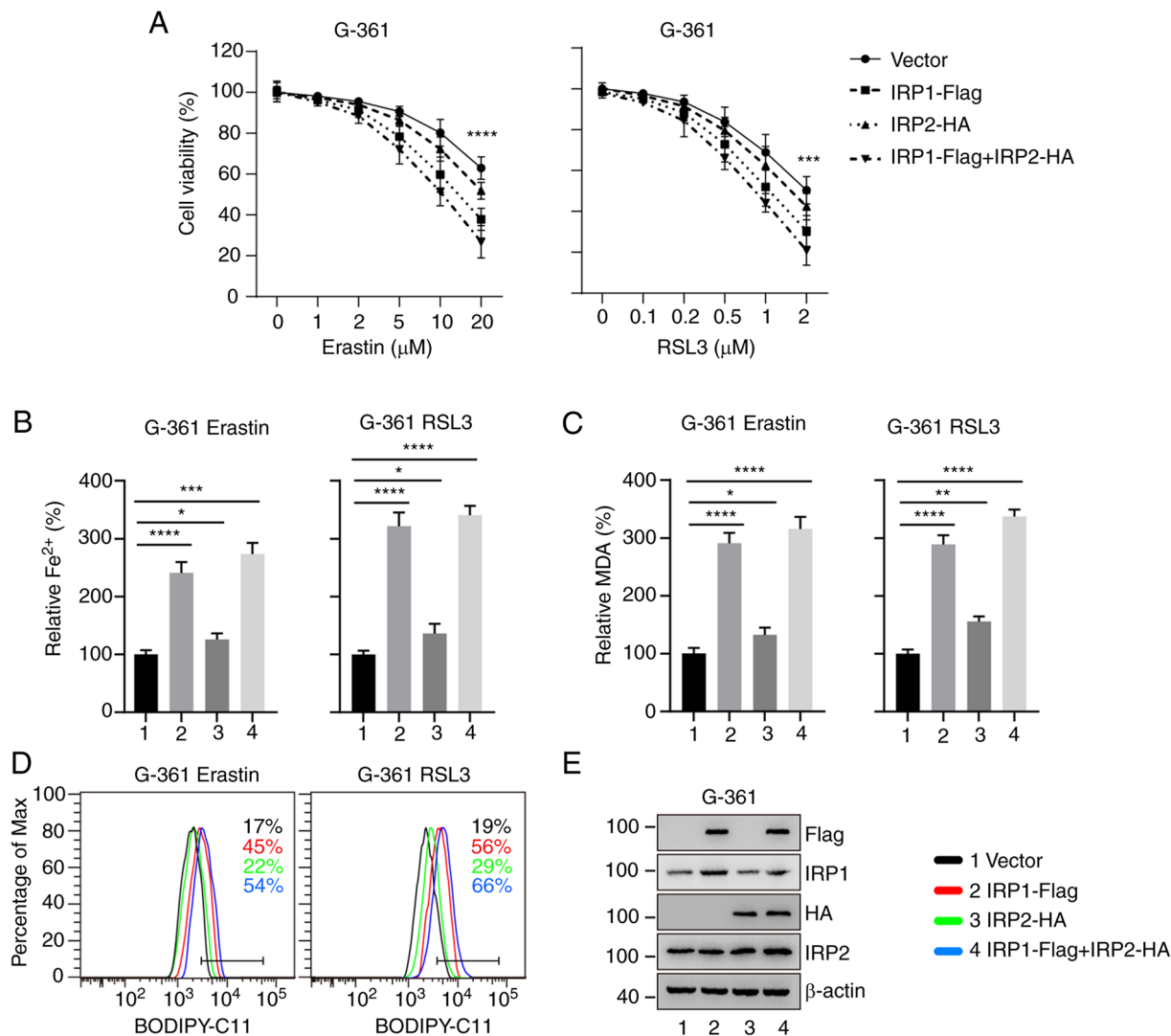


Figure S4. Erastin and RSL3 promote the transition of ACO to IRP1. (A) ACO activity decreased significantly in G-361 melanoma cells following erastin and RSL3 treatment. (B) Erastin and RSL3 promoted the IRE-binding activity of IRP1. (C) Erastin and RSL3 had no significant effect on the IRE-binding activity of IRP2. The relative abundances of IRP1-Flag and IRP2-Flag were analyzed by RNA immunoprecipitation. Data are presented as the mean \pm SD of three independent experiments. *** P <0.001; **** P <0.0001. ACO, aconitase; IRP, iron regulatory protein; IRE, iron-responsive element; sh, short hairpin RNA; n.s., not significant.

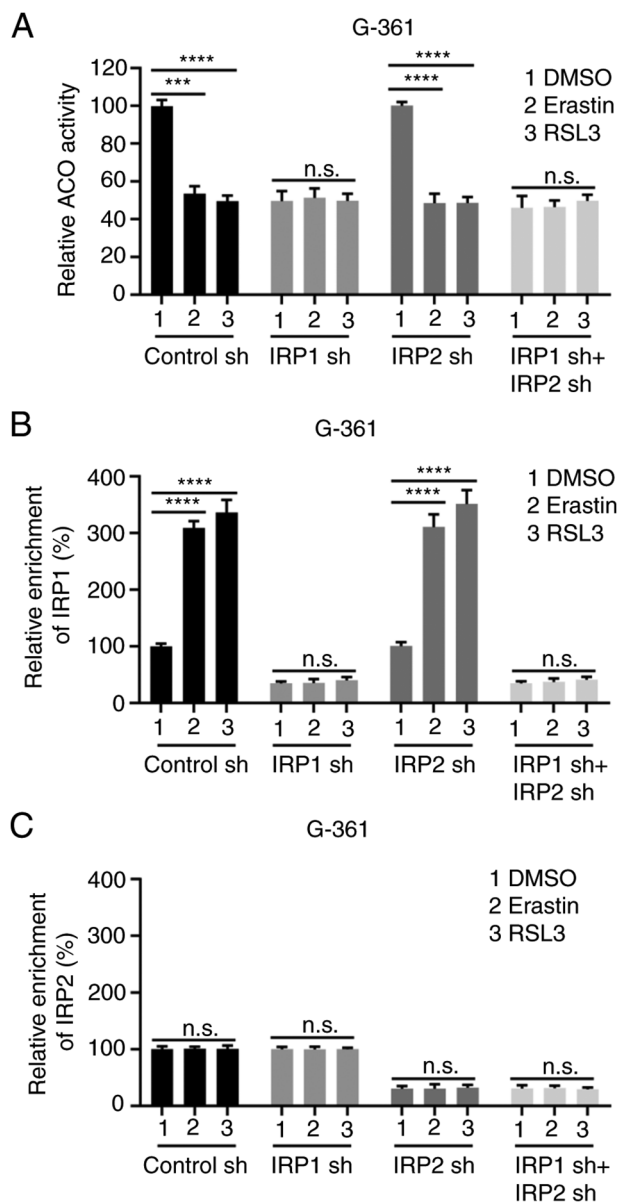


Figure S5. IRP1 is essential for the expression of iron-regulating proteins in ferroptosis. (A) Knockdown of IRP1 suppressed the mRNA expression levels expression of TFRC in G-361 melanoma cells following erastin and RSL3 treatment. (B and C) Knockdown of IRP1 had no significant effect on the mRNA expression levels of (B) FPN and (C) FTH1 following erastin and RSL3 treatment. (D) Protein levels of TFRC, FPN and FTH1 in G-361 cells treated with erastin or RSL3. Data are presented as the mean \pm SD of three independent experiments. *** P <0.001. IRP, iron regulatory protein; TFRC, transferrin receptor; FPN, ferroportin; FTH1, ferritin heavy chain 1; sh, short hairpin RNA; n.s., not significant.

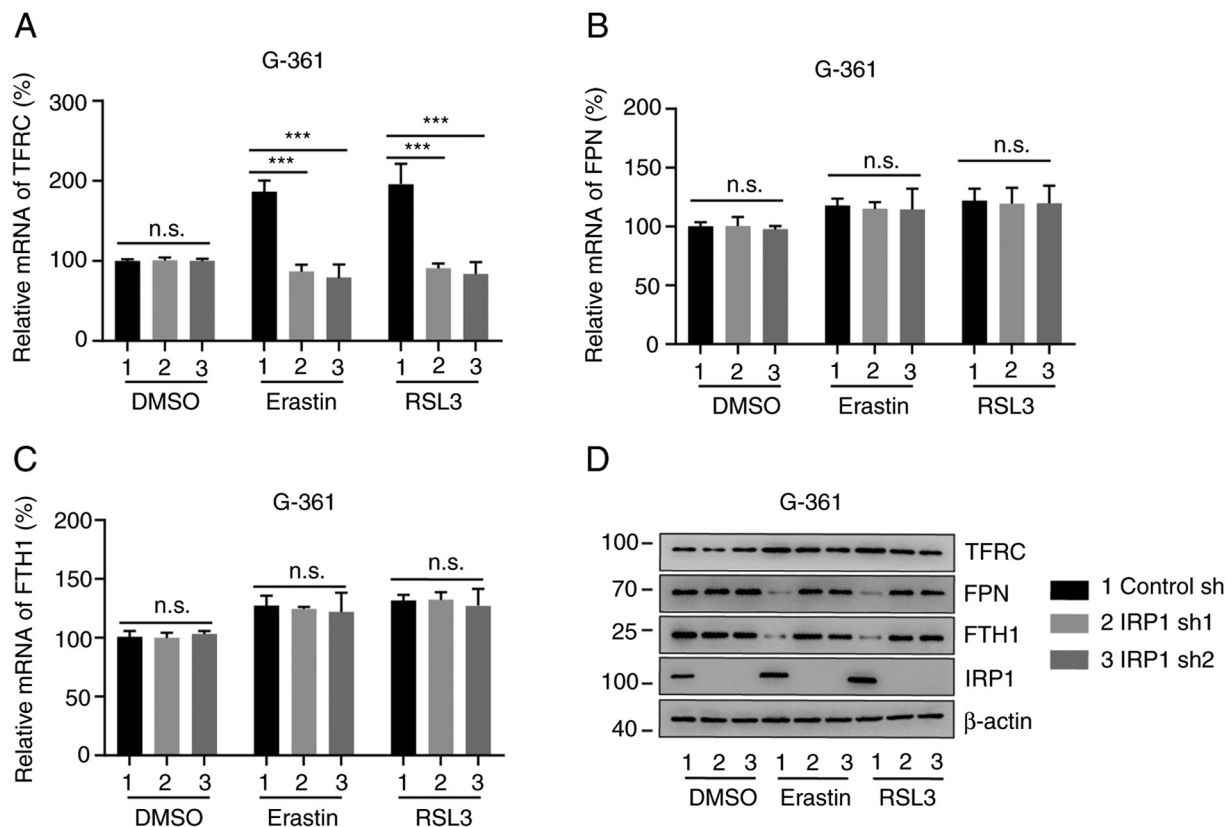


Figure S6. IRP1 function in ferroptosis depends on TFRC, FPN and FTH1. (A) Overexpression of TFRC and knockdown of FPN and FTH1 enhanced erastin- and RSL3-induced ferroptotic cell death in IRP1 knockdown G-361 melanoma cells. (B) Overexpression of TFRC and knockdown of FPN and FTH1 promoted erastin- and RSL3-induced iron accumulation in IRP1 knockdown G-361 melanoma cells. (C and D) Overexpression of TFRC and knockdown of FPN and FTH1 promoted erastin- and RSL3-induced (C) MDA accumulation and (D) lipid ROS accumulation in IRP1 knockdown G-361 melanoma cells. (E) Protein levels of IRP1, TFRC, FPN and FTH1 in G-361 cells transfected with the indicated constructs. (F) TFRC-Flag, FPN sh and FTH1 sh constructs were co-transfected in A375 and G-361 cells treated with erastin or RSL3, and the protein levels of TFRC, FPN, and FTH1 were detected. Data are presented as the mean \pm SD of three independent experiments. * P <0.05; ** P <0.01; *** P <0.001. IRP, iron regulatory protein; TFRC, transferrin receptor; FPN, ferroportin; FTH1, ferritin heavy chain 1; sh, short hairpin RNA; MDA, malondialdehyde; n.s., not significant.

