Figure S1. Western blotting of the target protein HES1 after transfection. (A and B) HES1 expression was detected using western blotting in H9c2 cells pretreated with pAD/HES1, pAD/HES1-shRNA, or NC. Data are presented as the mean ± SD (n=3). *P<0.05 and ***P<0.001. HES1, hairy, and enhancer of split 1; NC, negative control; shRNA, short hairpin RNA.

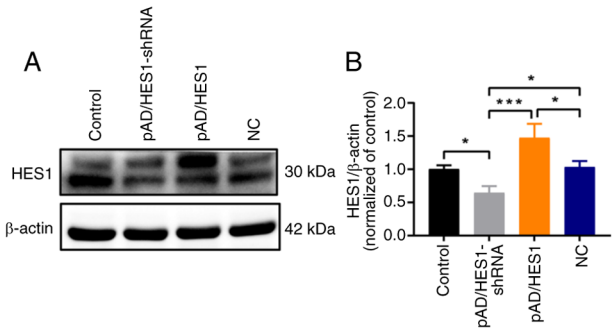

Figure S2. Cur pretreatment attenuates erastin- or A/R-induced ferroptosis. (A and F) Cell Counting Kit-8 assay for the viability of erastin- or A/R-induced H9c2 cardiomyocytes after Cur pretreatment. (B and G) LDH. (C and H) MDA. (D and I) SOD. (E and J) GSH/GSSG ratio. (K) Erastin- or A/R-induced cardiomyocyte viability after Cur pretreatment detected using western blotting analysis along with the expression of HES1 and ferroptosis-associated proteins in cells. (L and M) Flow cytometry M61 method for MPTP. Data are presented as the mean ± SD (n=3). *P<0.05, **P<0.01, ***P<0.001 and ****P<0.0001. A/R, anoxia/reoxygenation; MDA, malondialdehyde; SOD, superoxide dismutase; GSH/GSSH, glutathione/glutathione disulfide; HES1, hairy and enhancer of split 1; GPX4, glutathione peroxidase 4; MPTP, mitochondrial permeability transition pore.

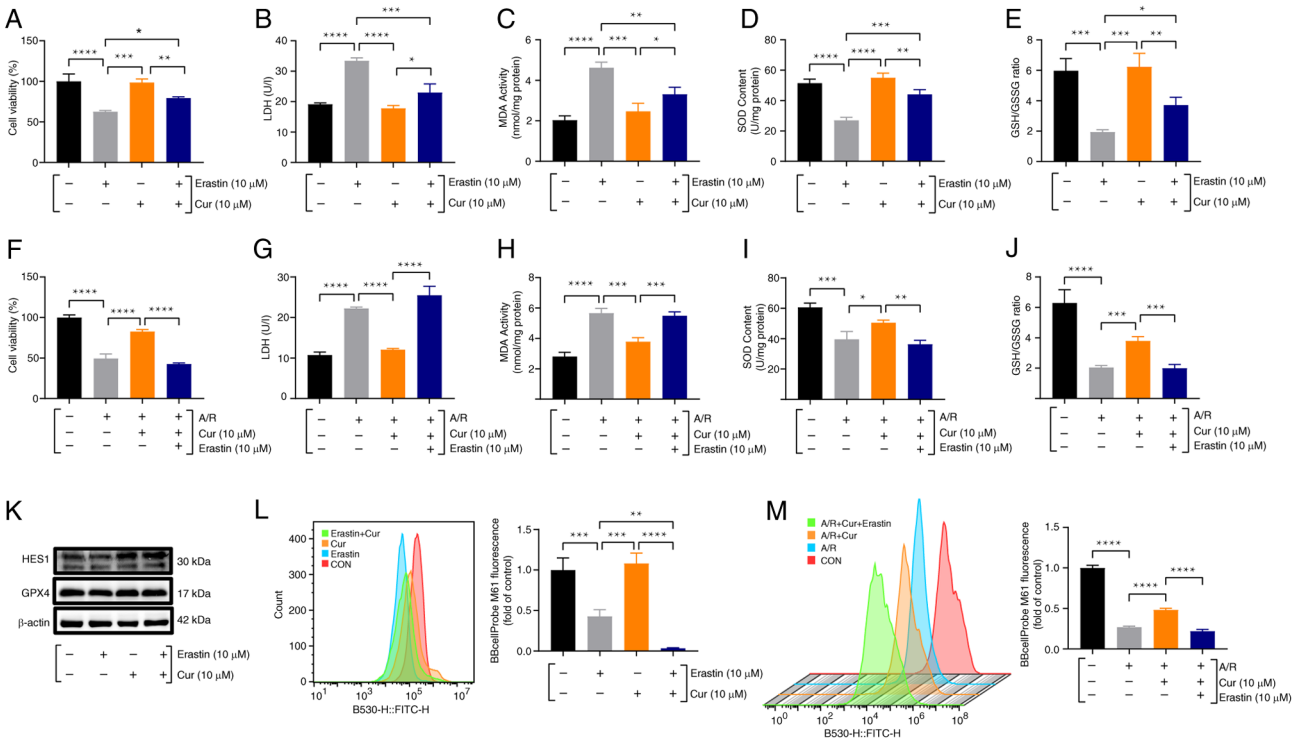

Figure S3. HES1 is involved in erastin- or A/R-induced ferroptosis bioprocess. (A and H) Cell Counting Kit-8 assay of A/R-induced H9c2 cardiomyocyte viability after pretreatment with pAD/HES1, pAD/HES1-shRNA, or NC. (B and I) LDH. (C and J) MDA. (D and K) SOD. (E and L) GSH/GSSG ratio. (F and M) Viability of H9c2 cardiomyocytes pretreated with pAD/HES1, pAD/HES1 shRNA, or NC pretreatment; HES1 and ferroptosis-related protein expression in erastin or A/R-induced cells was detected using western blot analysis. (G and N) MPTP was detected via the flow cytometry M61 method. Data are presented as the mean ± SD (n=3). *P<0.05, **P<0.01, ***P<0.001 and ****P<0.0001. HES1, hairy and enhancer of split 1; A/R, anoxia/reoxygenation; shRNA, short hairpin RNA; SOD, superoxide dismutase; MDA, malondialdehyde; GSH/GSSH, glutathione/glutathione disulfide; PTGS2, prostaglandin-endoperoxide synthase 2; GPX4, glutathione peroxidase 4; MPTP, mitochondrial permeability transition pore; NC, negative control.

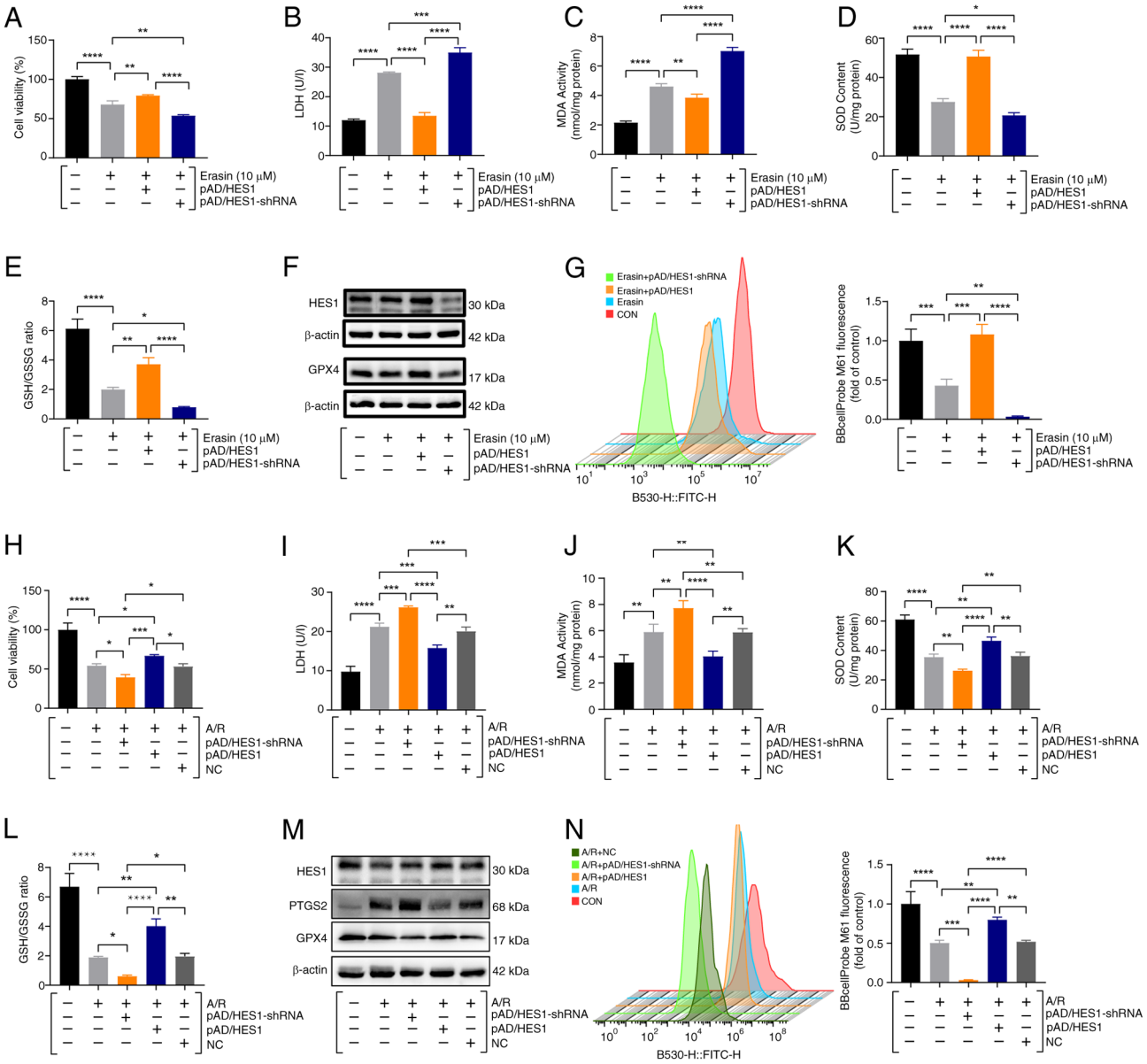

Figure S4. Cur pretreatment inhibits A/R-triggered excessive autophagy in cardiomyocytes via upregulating HES1. (A-F) Expression and quantification of HES1, P62, LC3II/I, PTGS2 and GPX4 proteins in A/R-triggered cells were determined using western blot analysis after pretreatment with Cur, RA and 3MA. Data are presented as the mean ± SD (n=3). *P<0.05, **P<0.01, ***P<0.001 and ****P<0.0001. Cur, curcumin; RA, rapamycin; 3MA, 3-methyladenine; A/R, anoxia/reoxygenation. HES1, hairy and enhancer of split 1; P62, Sequestosome 1; LC3II/I, microtubule-associated protein 1 light chain 3; ns, not significant.