Figure S1

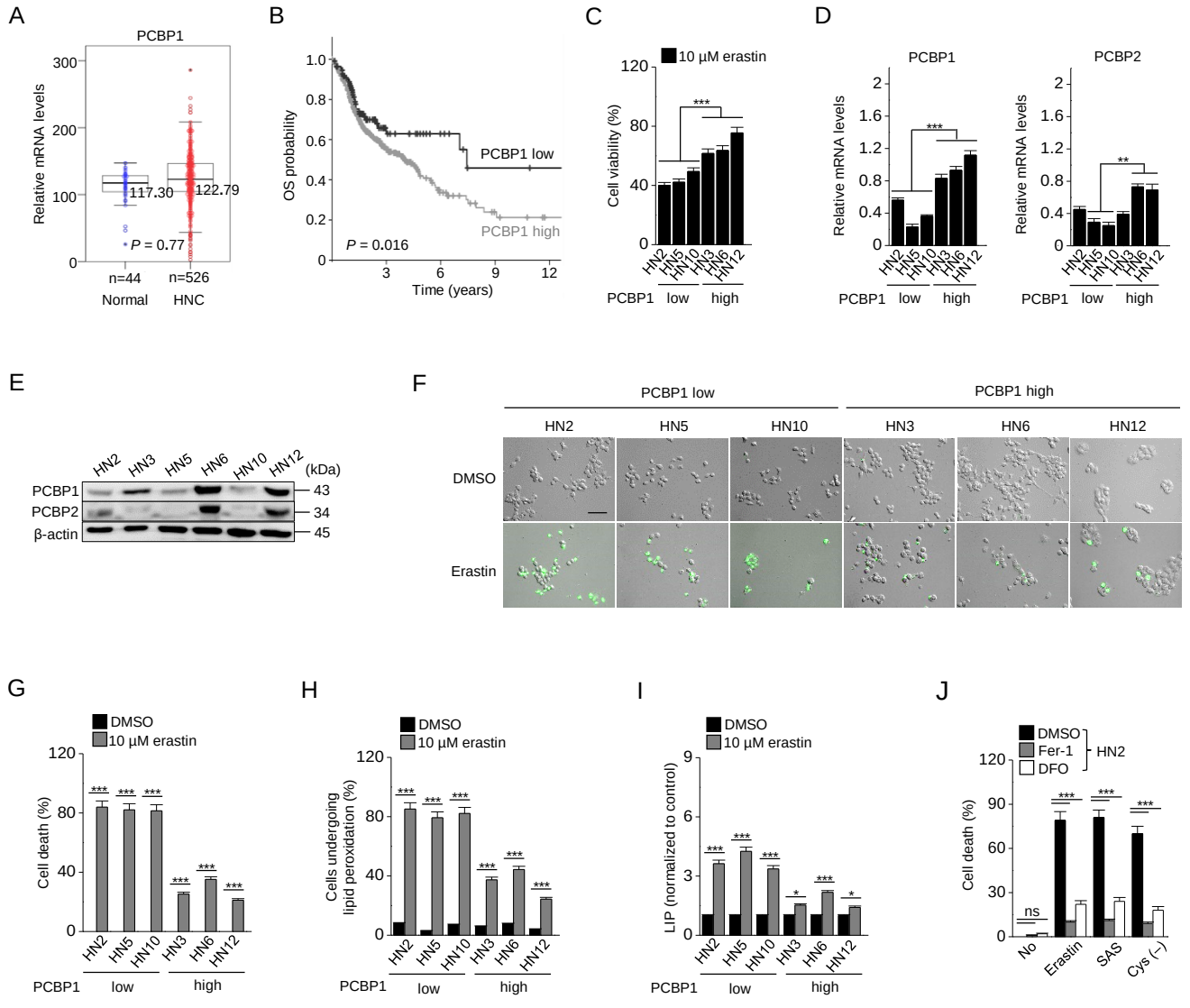

Fig. S1. PCBP1 expression is associated with HNC survival and ferroptosis sensitivity. (***A***) Comparison of PCBP1 mRNA expression levels between 44 normal mucosa samples and 526 HNC samples from the TCGA database. The bar values indicated median and quartiles. (***B***) Kaplan–Meier curves estimating overall survivals (OS) of patients with high and low PCBP1 expression in the HNC cohort of the TCGA datasets. Log-rank test, P <0.05. (***C***–***D***) Cell viability of HNC cell lines with exposure to 10 μM erastin (C) and PCBP1/2 mRNA expression (D). Cell viability was measured by cell counting kit-8 (CCK-8) after drug treatment for 48 h. The cell viability by erastin treatment was relative to the DMSO control (%). (***E***) Immunoblotting of PCBP1 and PCBP2 in HNC cell lines. (***F***–***I***) Cell death (F–G), lipid peroxidation (H), and liable iron pool (I) assays in cell lines exposed to dimethyl sulfoxide (DMSO) or 10 μM erastin for 48 h. Cell death was measured by 5 μM SYTOX Green after 10 μM erastin treatment for 48 h. Lipid peroxidation was measured using flow cytometry by adding 5 μM BODIPY C11 after 10 μM erastin treatment for 6 h. Labile iron pool (LIP) was assessed by 8 μg/ml calcein-AM staining after 10 μM erastin treatment for 6 h. These were quantified using ImageJ software. Original magnification, ×200. Scale bar, 50 μm. (***J***) Cell death in HN2 cells (B) treated with or without 10 μM erastin, 1 mM sulfasalazine (SAS), or cyst(e)ine deprivation (cys (–)) for 48 h. The cells were also co-treated with 2 μM ferrostatin-1 (Fer-1) or pretreated with 100 μM deferoxamine (DFO) for 12 h. The error bars represent standard errors from three independent experiments. ns; no significance, * P <0.05, ** P <0.01, *** P <0.001 among different cell lines or treatments.

Figure S2

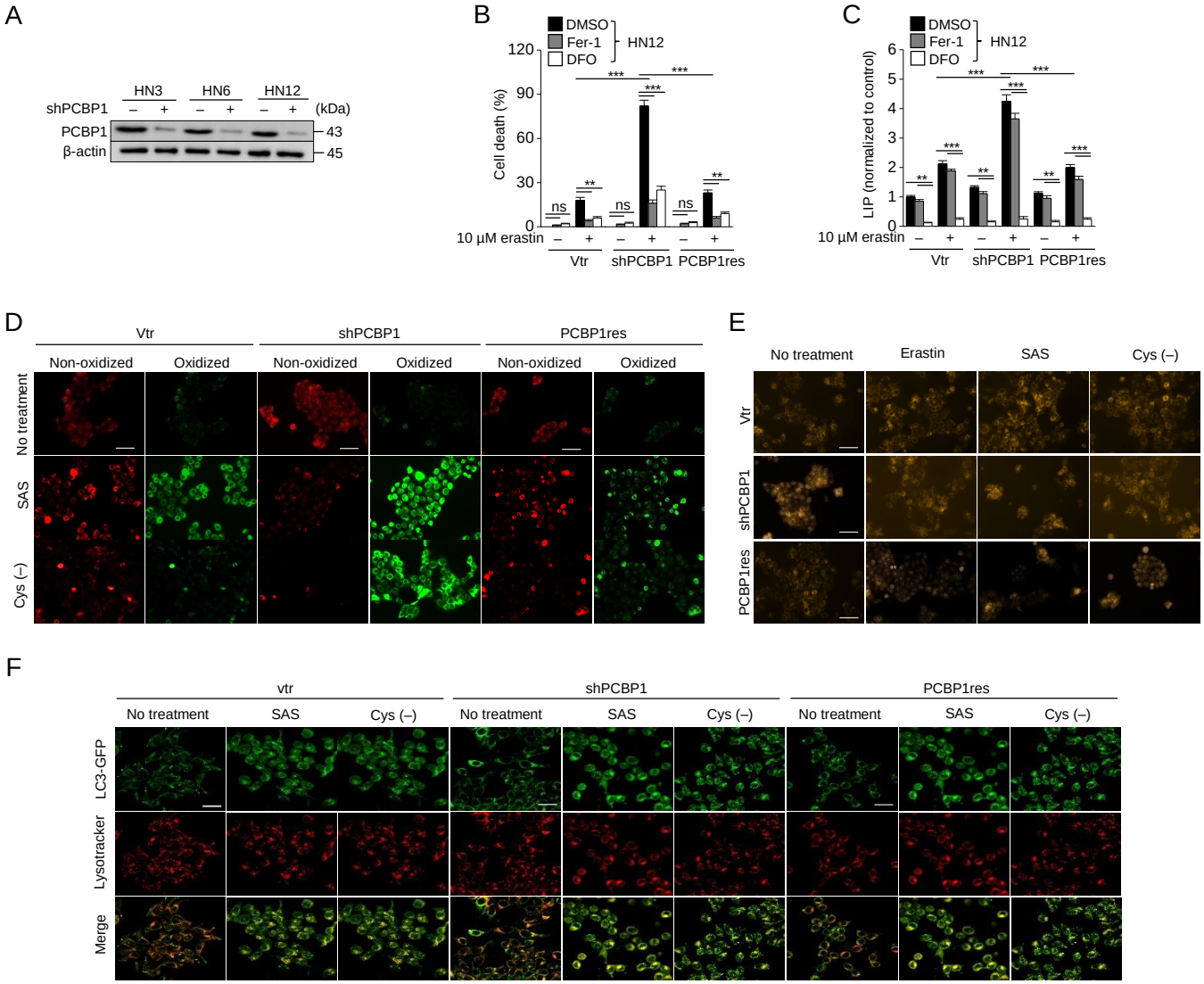

Fig. S2. Knockdown of PCBP1 promotes iron accumulation and lipid peroxidation. (***A***) PCBP1 knockdown was confirmed by immunoblotting in HN3, HN6, and HN12 cells with relatively high expression of PCBP1. (***B***–***C***) Cell death and liable iron pool assays were measured in HN12 cells (C–D) with vector (vtr), shPCBP1, or shPCBP1 plus PCBP1 overexpression (PCBP1 res) transfection exposed to 10 μM erastin for 48 h. The cells were also co-treated with 2 μM Fer-1 or pretreated with 100 μM DFO for 12 h. The error bars represent standard errors from three independent experiments. ns; no significance, ** P <0.01, *** P <0.001 among different treatments. (***D***) The fluorescence images by BOPDIPY^{TM} 581/591 C11 staining. Non-oxidized (red) and oxidized (green) were measured in HN12 cells with vector, shPCBP1, or PCBP1 res transfection exposed to 1 mM SAS or cyst(e)ine deprivation for 6 h and then add 5 μM BODIPY C11. (***E***) Cellular ferrous iron images using FerroOrange staining in HN12 cells with vector (vtr), shPCBP1, or PCBP1 res transfection after exposure to 10 μM erastin, 1 mM SAS or cyst(e)ine deprivation for 6 h. (***F***) The fluorescence image showing the co-localization of lysosomes and LC3-GFP. Original magnification, ×200. Scale bar, 50 μm. Fluorescence images were quantified by ImageJ.

Figure S3

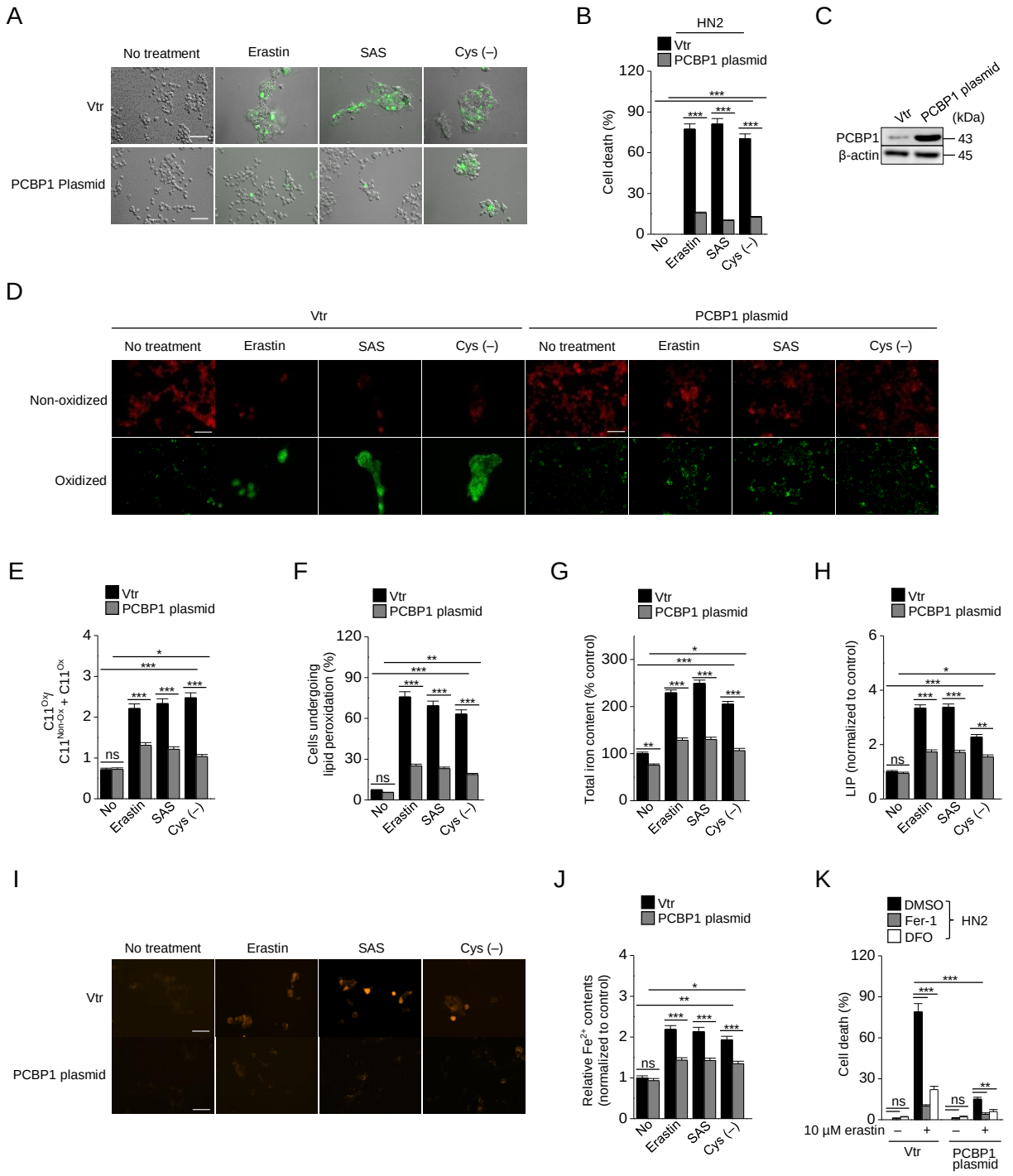

Fig. S3. PCBP1 overexpression inhibits ferroptotic cell death. (***A*–*B***) Cell death of HN2 cells with vector or PCBP1 cDNA transfection and treatment of 10 μM erastin, 1 mM SAS, or cyst(e)ine deprivation for 48 h. (***C***) PCBP1 overexpression was confirmed using immunoblotting. (***D*–*F***) Lipid ROS was measured by BODIPY C11 staining and FACS in HN2 cells after 10 μM erastin, 1 mM SAS, cyst(e)ine deprivation for 6 h. The fluorescence images were quantified using ImageJ (E). (***G*–*J***) Intracellular levels of total and ferrous iron were measured using iron assays (G) and FerroOrange (I–J) after 10 μM erastin, 1 mM SAS, and cyst(e)ine deprivation for 6 h. Labile iron pool (LIP) was measured by calcein-AM staining (H). The fluorescence images were quantified using ImageJ. (***K***) Cell death in HN2 cells with vector or PCBP1 cDNA transfection exposed to 10 μM erastin for 48 h. The cells were also co-treated with 2 μM Fer-1 or pretreated with 100 μM DFO for 12 h. The error bars represent standard errors from three independent experiments. ns; no significance, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ among different cell groups.

Figure S4

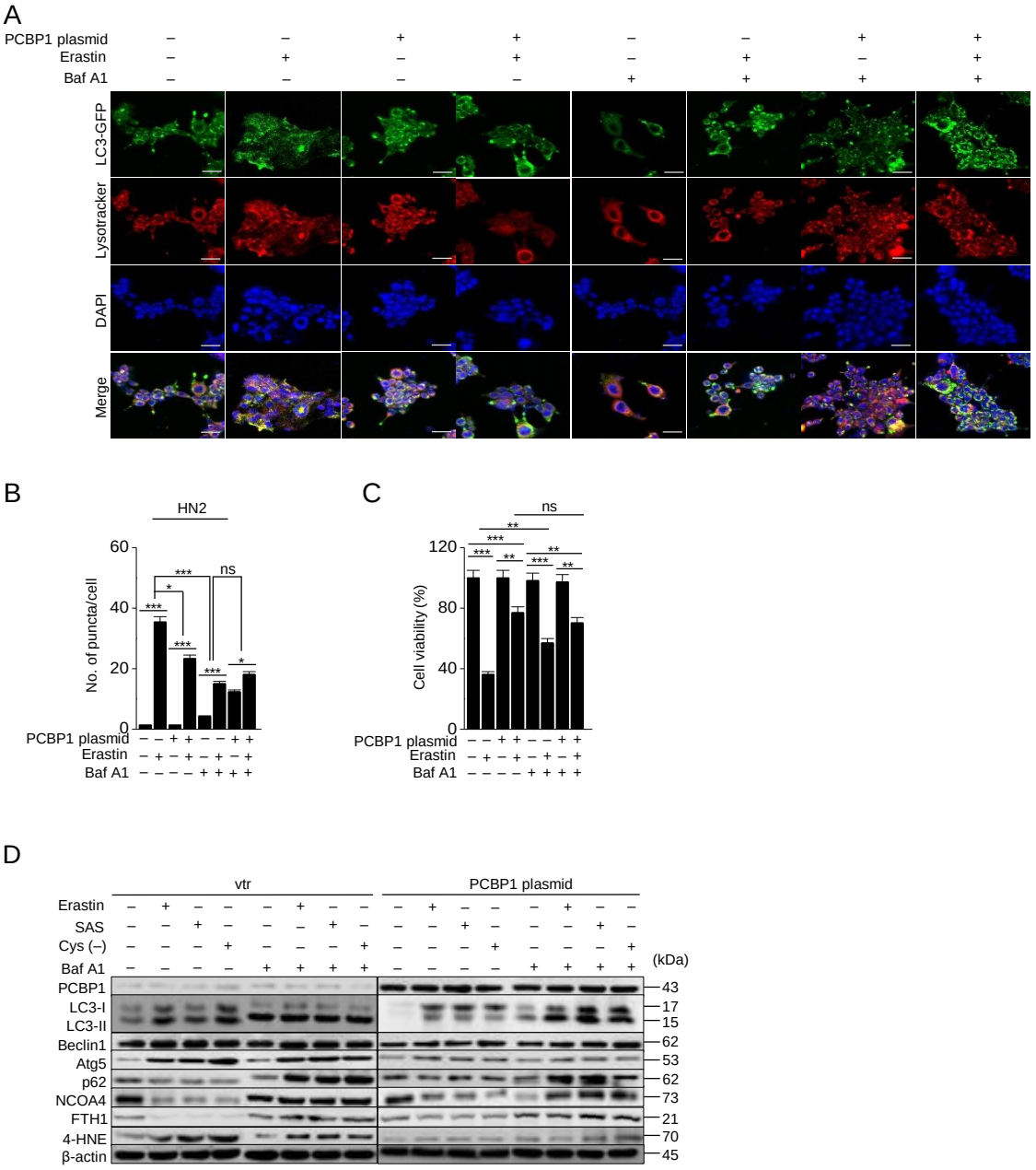

Fig. S4. PCBP1 overexpression inhibits ferritinophagy. (***A*–*B***) Measurements of LC3 puncta and lysosomes using immunofluorescence in HN2 cells with vector or PCBP1 cDNA transfection and treatment of 10 μM erastin treatment for 6 h, and then treated with or without 30 μM bafilomycin A1 for 2 h. Also, cell viability was measured after 48 h. The number of puncta was quantified by ImageJ (B). Original magnification, ×400. Scale bar, 25 μm. The error bars represent standard errors from three independent experiments. ns; no significance, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ among different cell groups. (***C***) Autophagic flux and ferroptotic cell death were determined by immunoblotting.

Figure S5

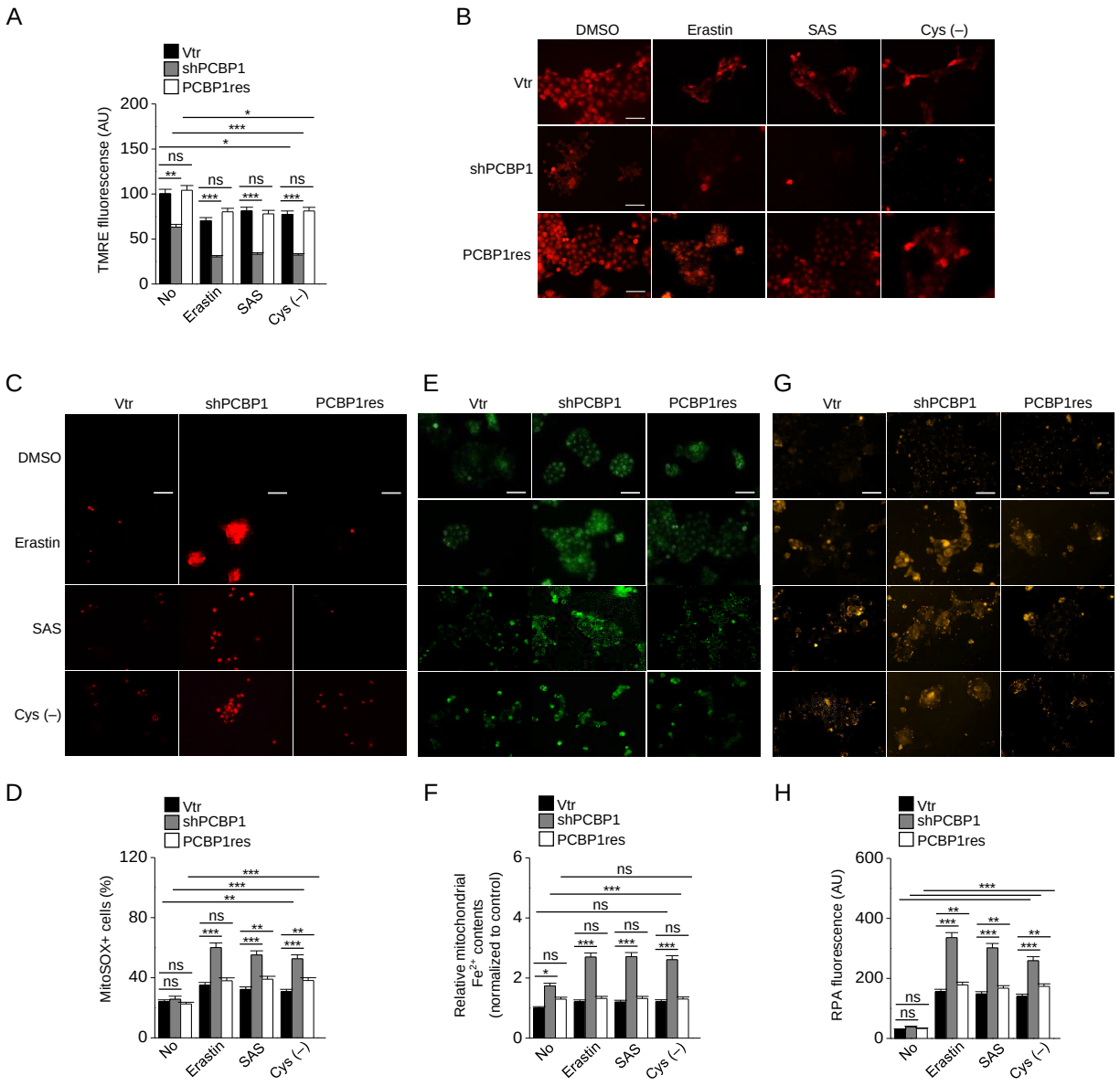

Fig. S5. Iron overload by enhanced ferritinophagy induces mitochondrial dysfunction. (***A–B***) Mitochondrial membrane potentials were measured by 200 nM tetramethylrhodamine ethyl ester (TMRE) after 10 μM erastin, 1 mM SAS, or cyst(e)ine deprivation treatment for 6 h. Each TMRE-stained fluorescence image was quantified by Image J. (***C–D***) Each cell was stained using MitoSOX to measure mitochondrial ROS after exposure to ferroptosis inducers for 6 h. MitoSOX fluorescence and positive cell fractions were measured by immunofluorescence and FACS, respectively. (***E–F***) Mitochondrial ferrous iron was determined by Mito-FerroGreen staining after exposure to ferroptosis inducers for 6 h. (***G–H***) Rhodamine B-[(1,10-phenanthroline-5-yl)-aminocarbonyl]benzyl ester (RPA) staining after exposure to ferroptosis inducers for 6 h. The fluorescence image was quantified by ImageJ. Original magnification, ×200. Scale bar, 50 μm. The error bars represent standard errors from three independent experiments. ns; no significance, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ between different cell groups.

Figure S6

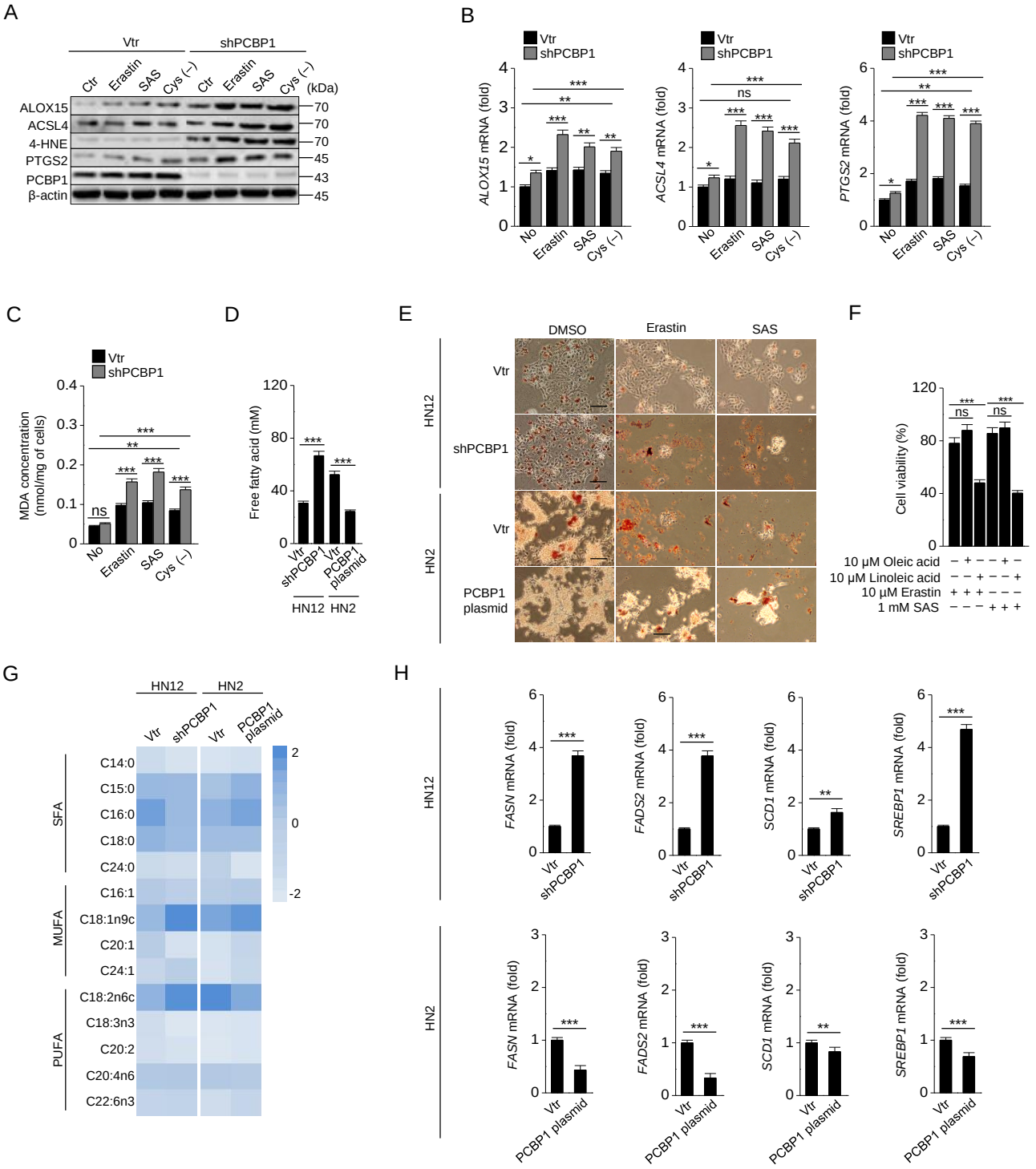

Fig. S6. PCBP1 negatively regulates lipid peroxidation. (***A–B***) Immunoblotting (A) and RT-qPCR (B) of ALOX15, ACSL4, and PTGS2 in HN12 cells with vector or shPCBP1 transfection after 10 μM erastin, 1 mM SAS, cyst(e)ine deprivation for 6 h. PCBP1 and 4-HNE were also immunoblotted. (***C***) The cellular level of malondialdehyde (MDA) was measured in HN12 cells with or without PCBP1 knockdown after exposure of ferroptosis inducers for 6 h. (***D–E***) Free fatty acid (FFA) level (D) and oil red staining (E) were measured in HN12 cells with or without PCBP1 knockdown and HN2 cells with or without PCBP1 overexpression. Scale bar, 50 μm. (***F***) Cell viability was measured after HN12 cells were treated with 10 μM erastin, 1 mM SAS combined with or without oleic acid (10 μM) or linoleic acid (10 μM) for 48 h. (***G***) Analysis of cellular lipid contents was performed using a FAME method by GC/MS in HN12 cells with or without PCBP1 knockdown and HN2 cells with or without PCBP1 overexpression. (***H***) RT-qPCR of FASN, FADS2, SCD1, and SREBP1 in HN12 and HN2 cells. The error bars represent standard errors from three independent experiments. ns; no significance, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ between different cell groups.

Figure S7

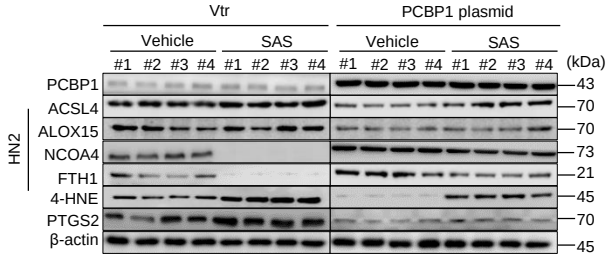

Fig. S7. The immunoblotting of PCBP1, ACSL4, ALOX15, NCOA4, FTH1, 4-HNE, and PTGS2, in HN2 tumors with vector (vtr) or PCBP1 plasmid transplanted in the nude mice and treatment with or without sulfasalazine (SAS).