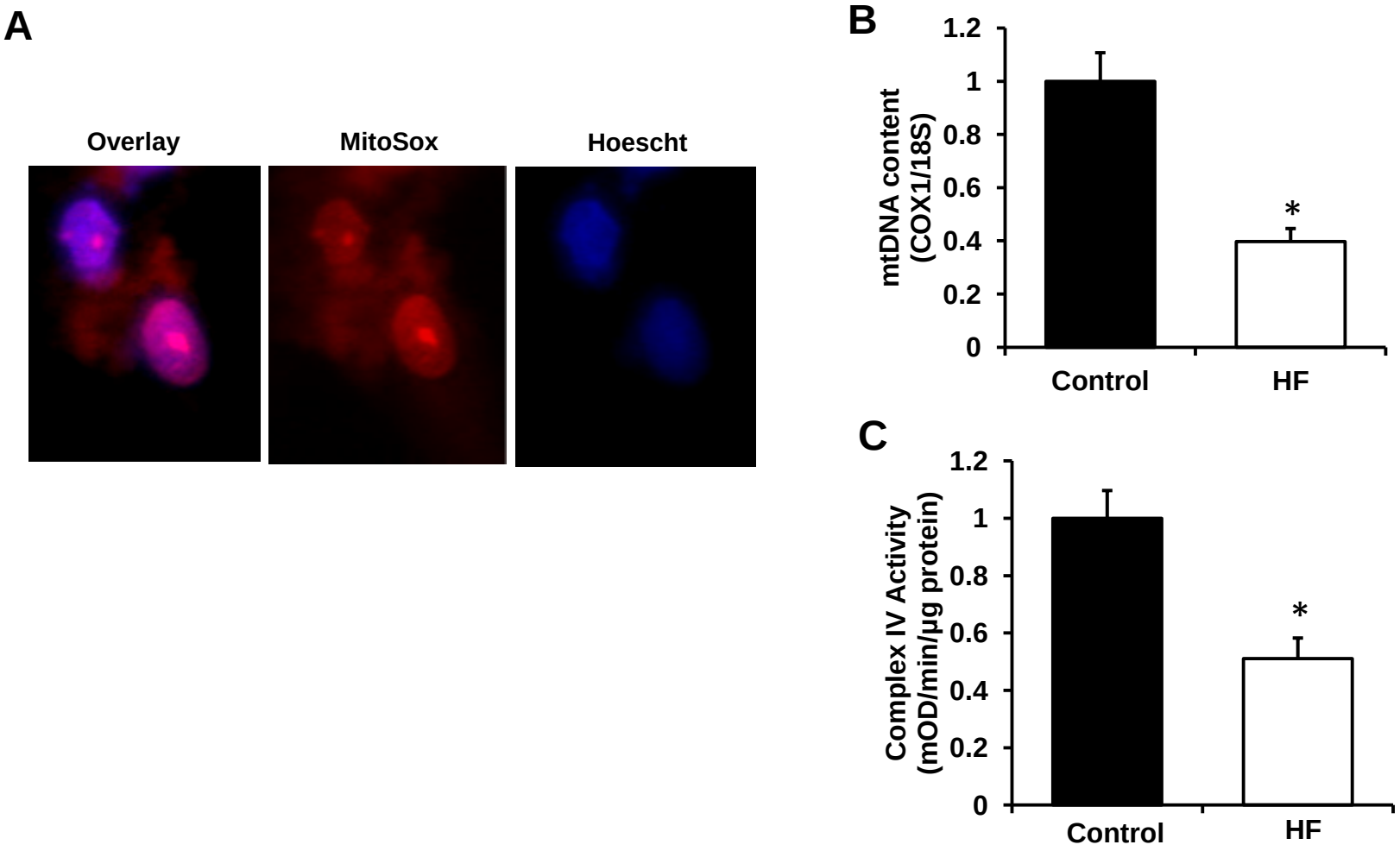
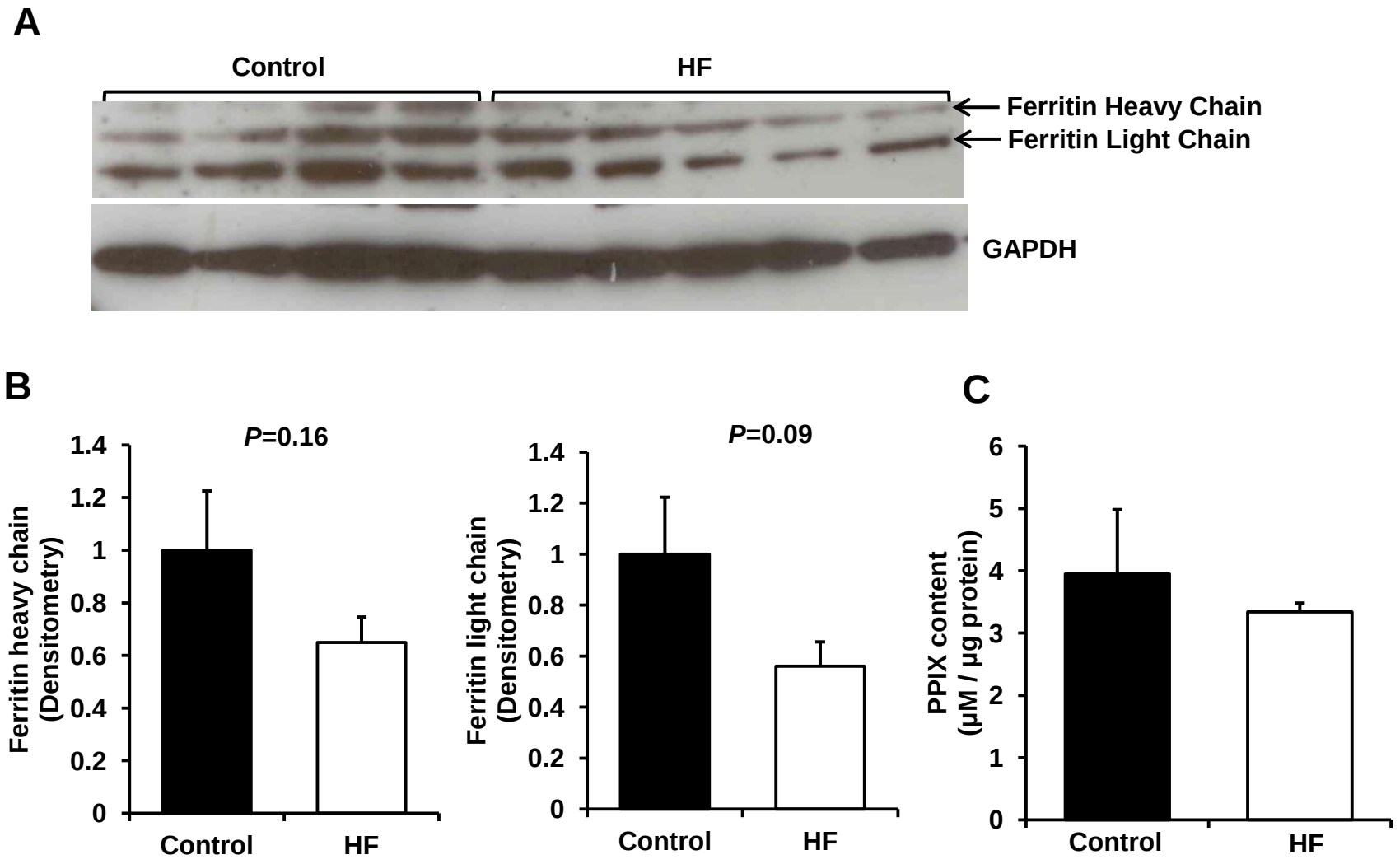
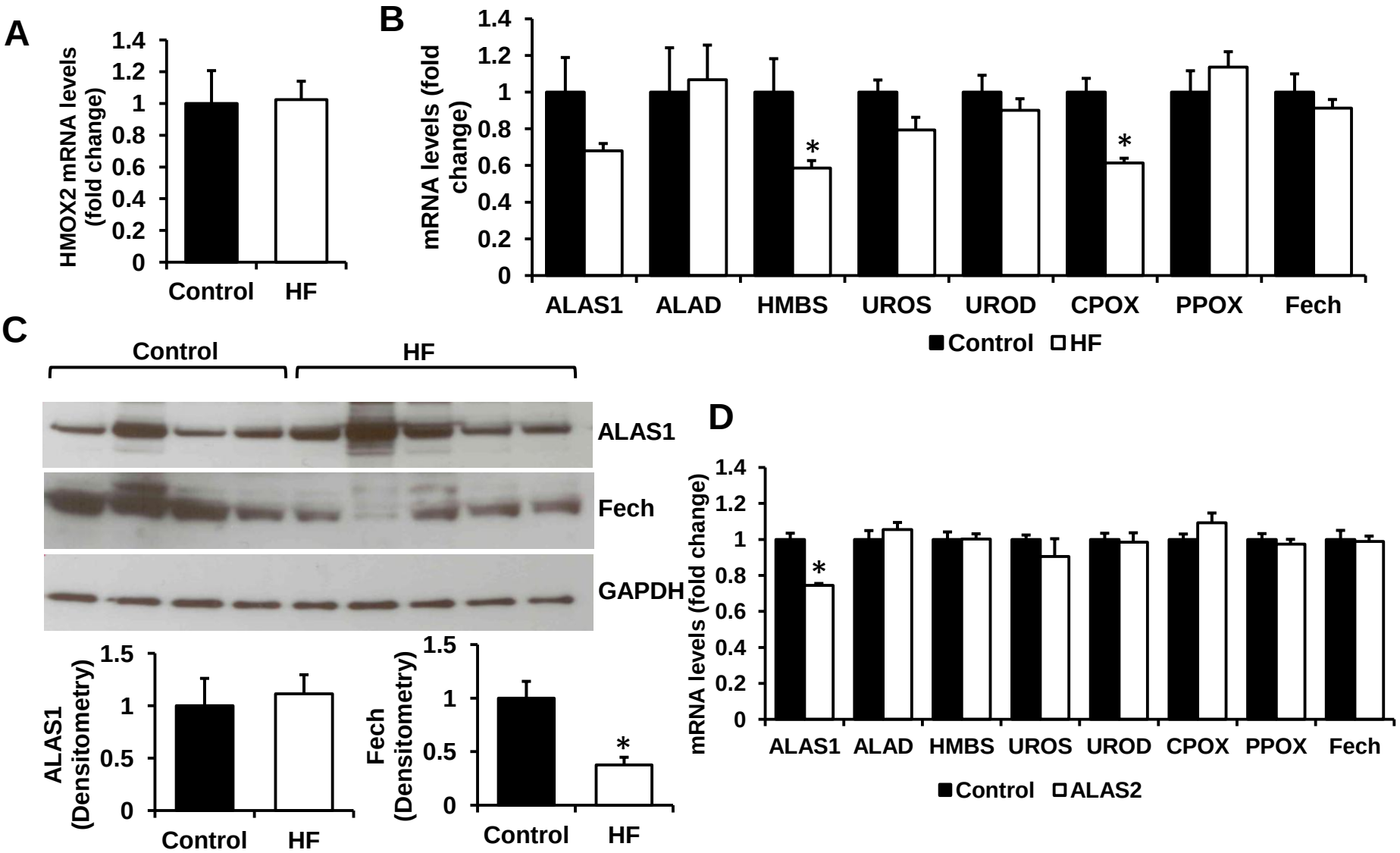


Supplemental Figure 1: Mitochondrial dysfunction in failing hearts
(A) Representative examples of MitoSox and Hoescht staining in H9c2 cells, 40X. (B) MtDNA content determined by qRT-PCR as a relative expression of mitochondria-encoded COX1 to nuclear 18S gene (n=10). (C) Activity of mitochondrial complex IV in failing hearts (n=10). Data are presented as mean ± SEM. * $p<0.05$ vs. control.

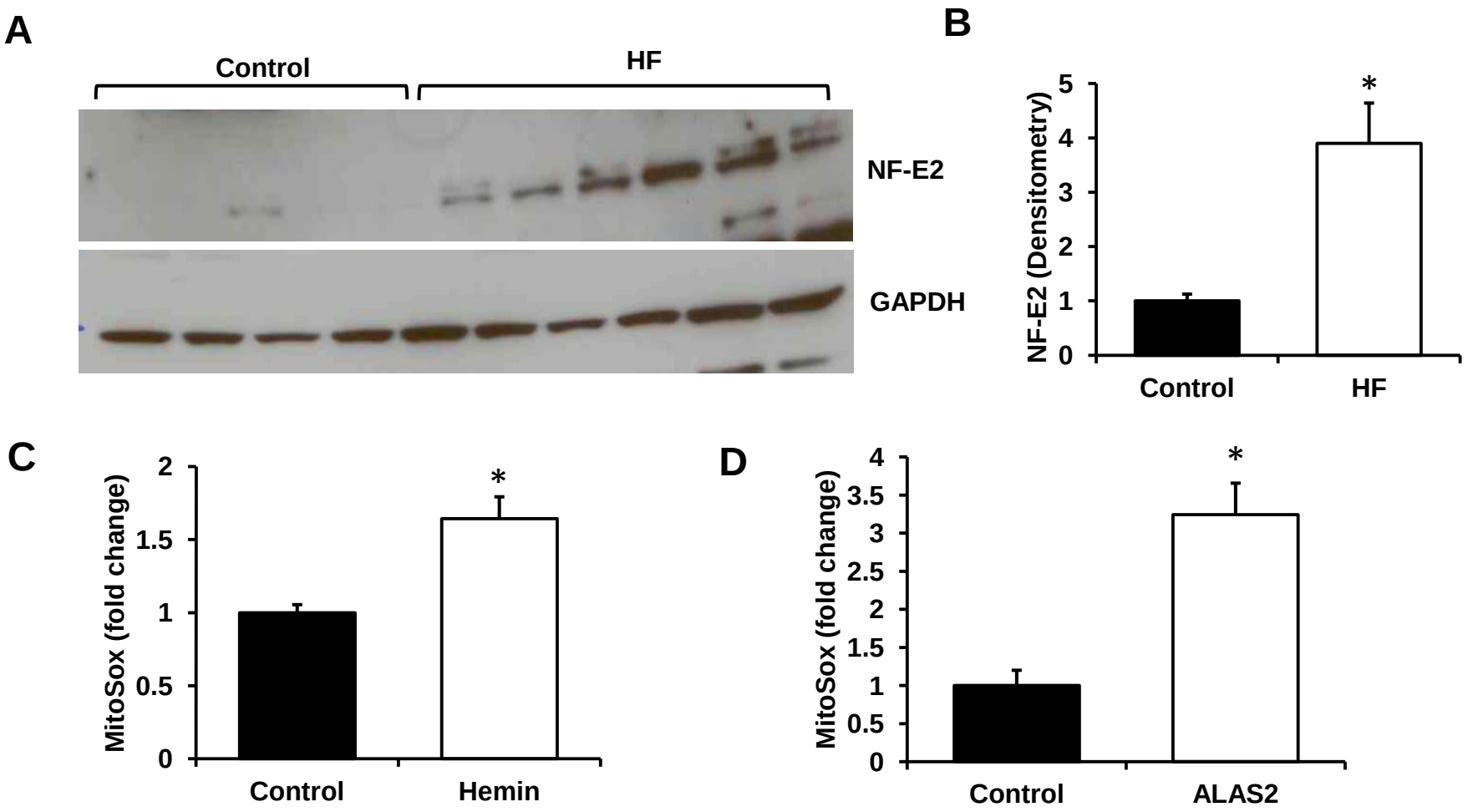


Supplemental Figure 2: Ferritin and protoporphyrin IX levels in failing hearts
(A) Western blot analysis of ferritin heavy and light chain expression in failing hearts. (B) Densitometry analysis of the Western blot in A (n=4-5). (C) PPIX levels in HF (n=6). Data are presented as mean ± SEM.

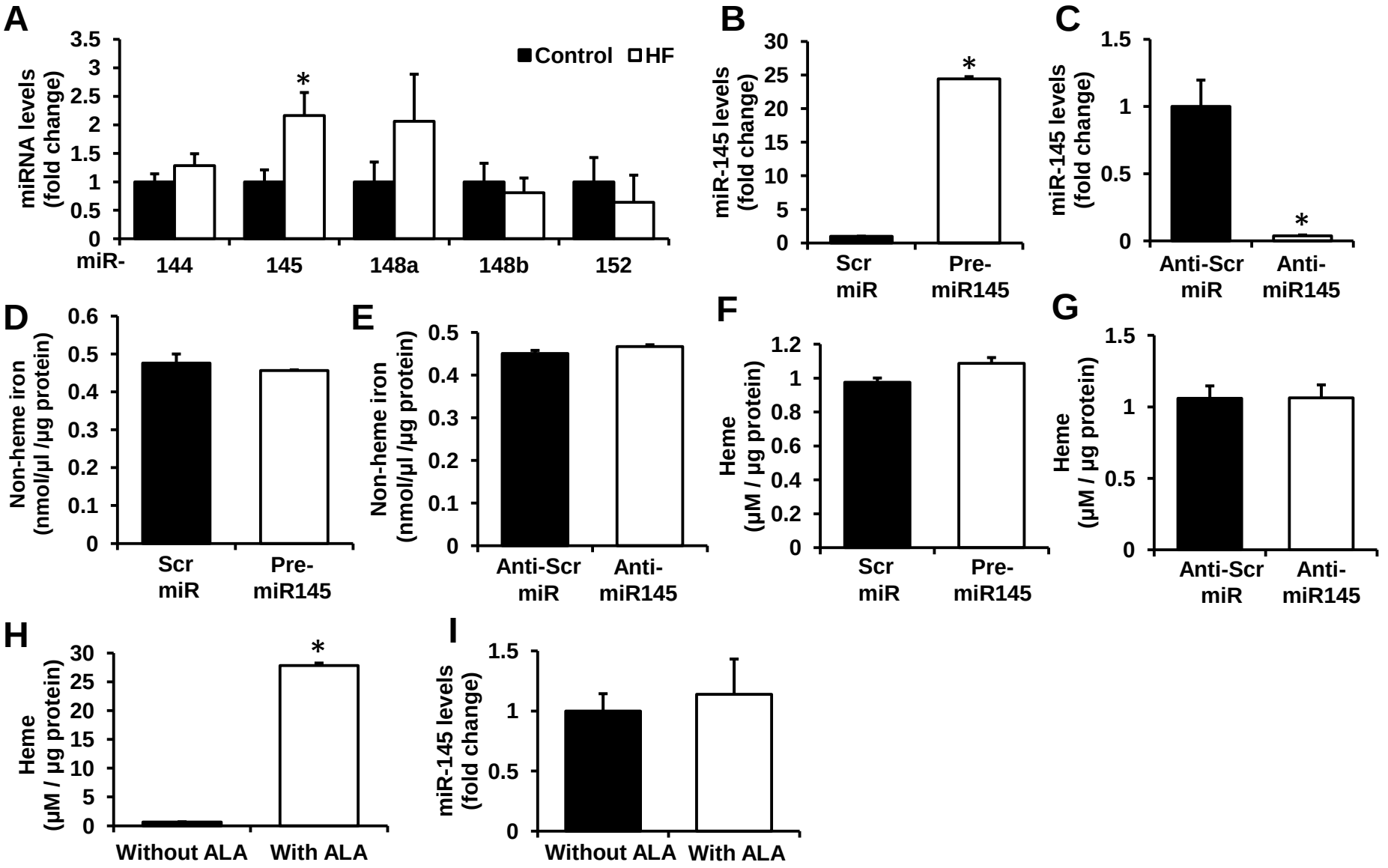


Supplemental Figure 3: Heme synthesis and degradation pathways in failing hearts

(A) mRNA levels of HMOX2 (n=10). (B) mRNA levels of heme synthesis genes (n=6). (C) Western blot of ALAS1 and ferrochelatase (Fech) catalyzing the first and the last steps of heme biosynthesis, respectively. Densitometry analyses are shown below the blots (n=4-5). (D) Expression of heme synthesis genes in H9c2 following ALAS2 overexpression (n=6). Data are presented as mean ± SEM. * $p<0.05$ vs. control. ALAD, delta-aminolevulinic acid dehydratase; HMBS, hydroxymethylbilane synthase; UROS, uroporphyrinogen III synthase; UROD, uroporphyrinogen decarboxylase; CPOX, coproporphyrinogen III oxidase; PPOX, protoporphyrinogen oxidase; Fech, ferrochelatase.



Supplemental Figure 4: NF-E2 and ROS.
(A) Western blot analysis of NF-E2 expression in failing hearts. (B) Densitometric analysis of the blot in A (n=4-5). (C) and (D) Quantification of total cellular MitoSox fluorescence without subtraction of nuclear fluorescence in H9c2 treated with hemin (C, n=4) or overexpressing ALAS2 (D, n=6). Data are presented as mean ± SEM. * $p<0.05$ vs. control.



Supplemental Figure 5: miR145 does alter heme in HF.
(A) Expression in control and failing hearts of microRNAs whose target sequences were identified by computational analysis of the 3'UTR of transferrin receptor 1 (n=10). Overexpression (B) and knockdown (C) of miR145 in H9c2 using pre-miR and anti-miR, respectively (n=3). (D) Non-heme iron levels in H9c2 with miR145 overexpression and (E) knockdown (n=3). (F) Heme iron levels in H9c2 with miR145 overexpression and (G) knockdown (n=3). (H) Heme levels and miR145 expression (I) in H9c2 treated with ALA (n=6). Data are presented as mean ± SEM. * $p<0.05$ vs. control. Scr, scrambled.