Expanded View Figures

Figure EV1. Activation of PPARα suppresses iron overload-induced Ferroptosis *in vivo*.

A Serum ALT and AST levels was measured in 8-week-old WT mice that were received intraperitoneal injections of dextriferron with or without GW7647 treatment.
B Liver sections were obtained from the indicated mice and stained with H&E. All scale bars are 50 μm.
C Liver sections were obtained from the indicated mice and stained with TUNEL. All scale bars are 20 μm.
D Hepatic and serum HMGB1 content were measured in the indicated mice.
E Hepatic Gpx4 mRNA levels were measured in the indicated mice.
F Hepatic MDA content was measured in 8-week-old WT mice that were received intraperitoneal injections of dextriferron with or without GW7647 treatment.
G Hepatic iron content was measured in 8-week-old WT mice that were received intraperitoneal injections of dextriferron with or without GW7647 treatment.

Data information: In (A–G), $n = 3$–5 mice/group. mRNA levels were normalized to 36B4 and are expressed relative to the mean value of the WT group; Data are presented as means ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$, determined by ANOVA.

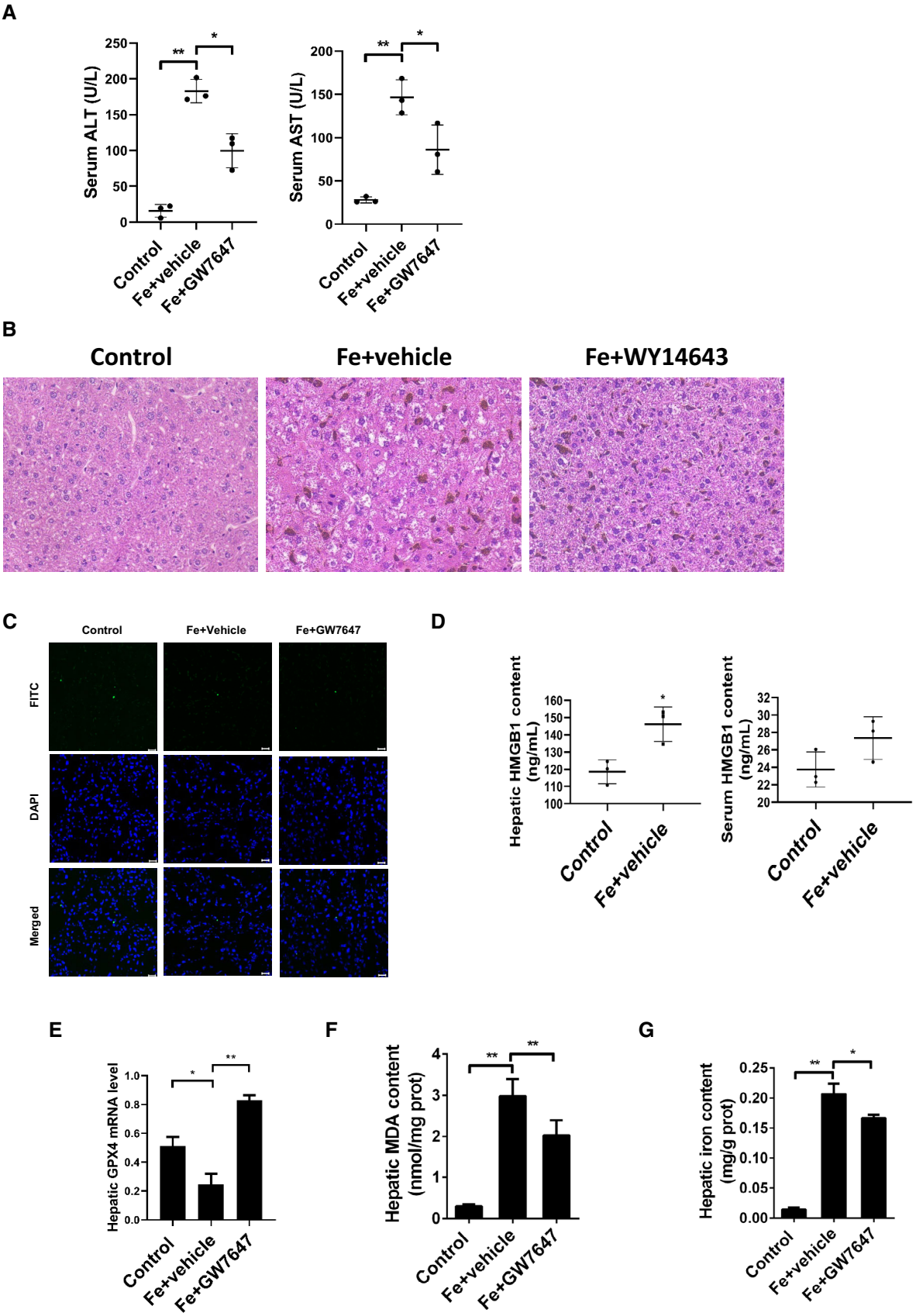

Figure EV1.

Figure EV2. Activated PPARα inhibited Ferroptosis caused by iron overload.

A Hepatic FSP1 mRNA levels were measured in the indicated mice.
B Hepatic MDM2 and MDMX mRNA levels were measured in the indicated mice.
C MDM2, MDMX, CPT1 and GPX4 mRNA levels were measured in the Hep1-6 cells after MDM2 or MDMX knockdown.
D Hepatic NRF2 mRNA levels, hepatic HO1 mRNA levels, and hepatic NQO1 mRNA levels were measured in the indicated mice.
E Liver sections were obtained from the indicated mice and stained with Prussian blue All scale bars are 50 μm.
F Hepatic iron content was measured in 8-week-old WT, PPARα$^{-/-}$ mice that were fed a HID or received intraperitoneal injections of dextriferron; n = 3–5 mice/group.

Data information: In (A, B, D-E), n = 3–5 mice/group. mRNA levels were normalized to 36B4 and are expressed relative to the mean value of the WT group; Data are presented as means ± SD. *P < 0.05, **P < 0.01, determined by ANOVA.

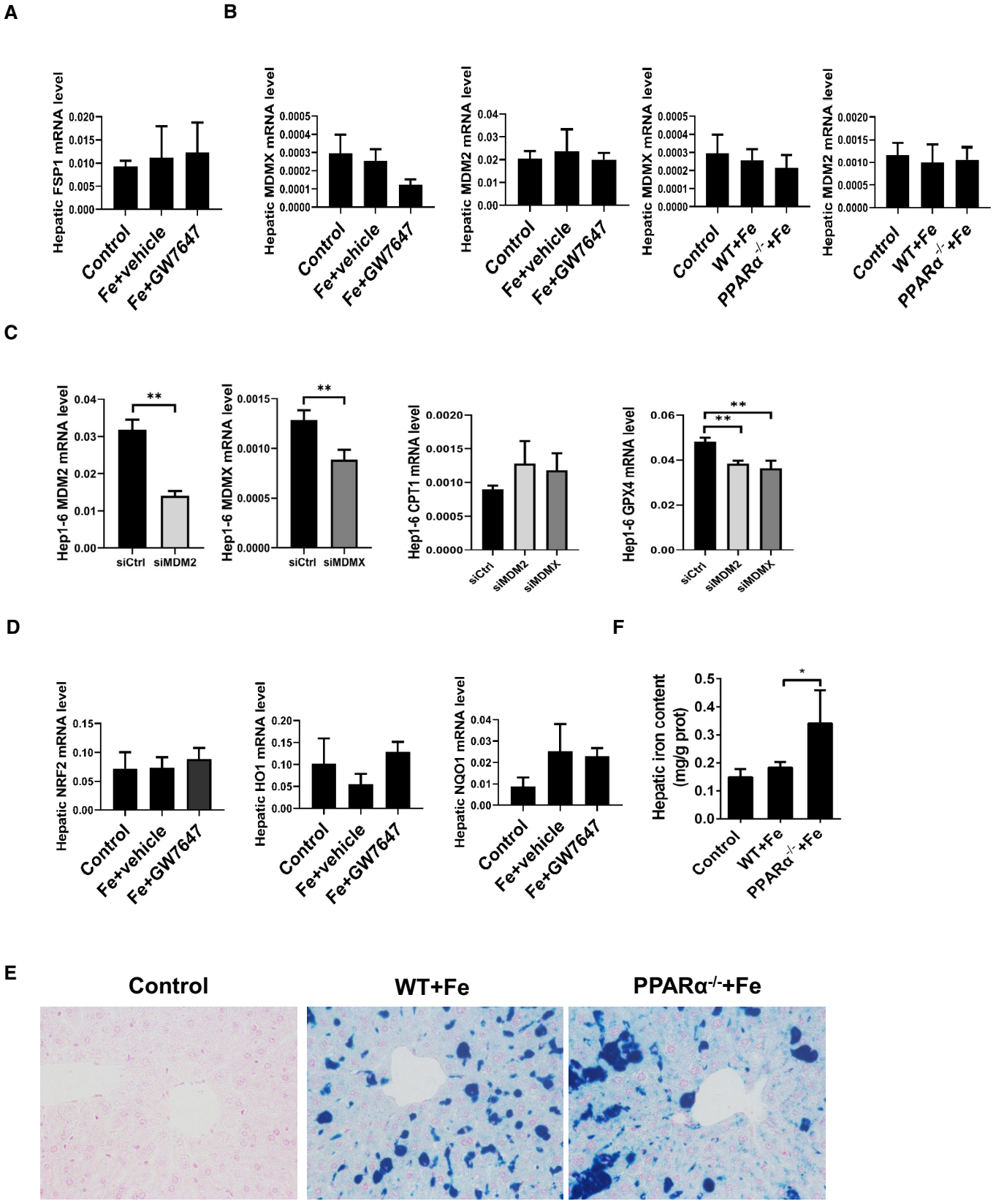

Figure EV2.

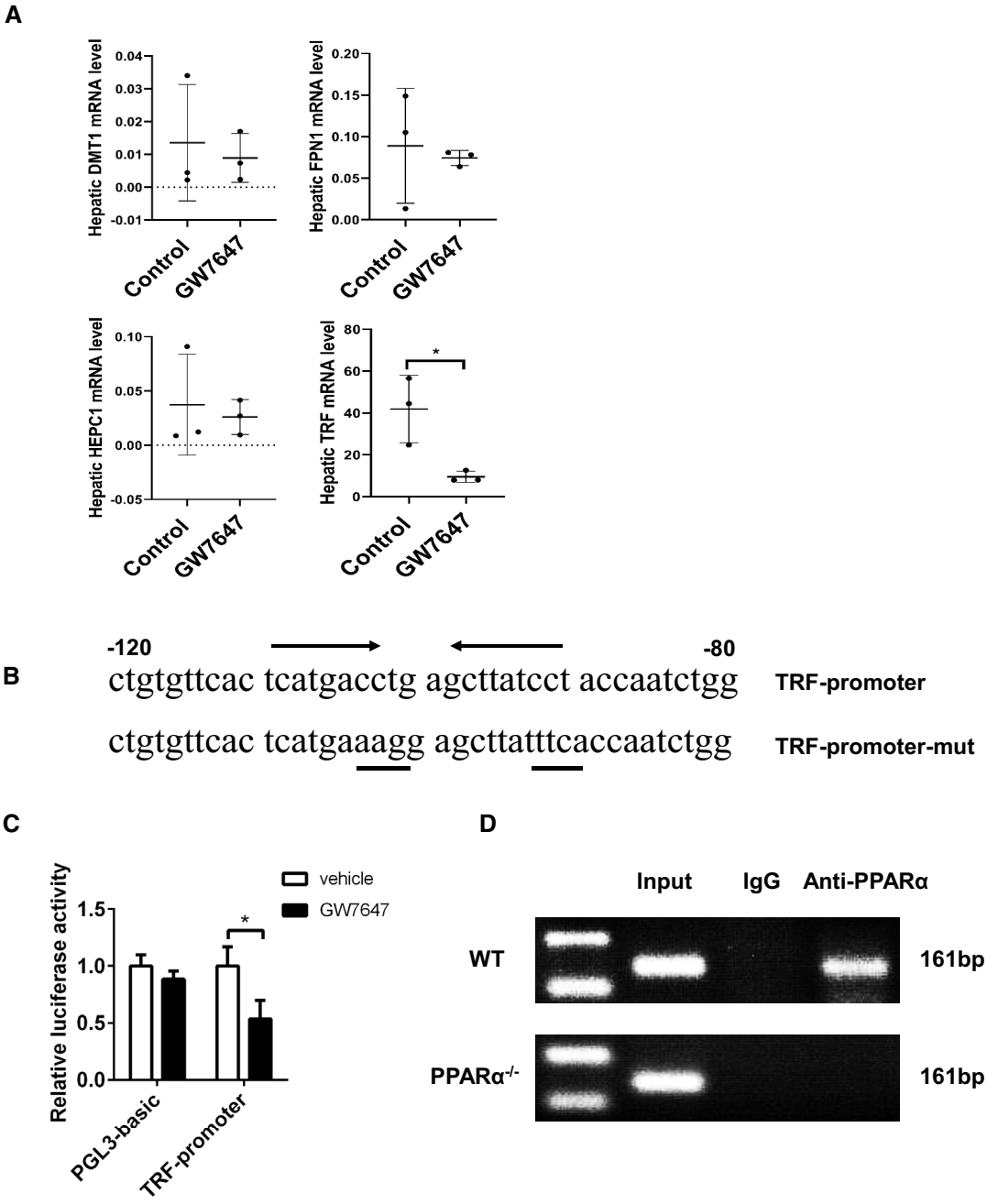

Figure EV3. PPARα regulates TRF transcriptional activity by binding to a PPRE in promoter of TRF.

A Hepatic DMT1, FPN1, HEPC1 and TRF mRNA levels were measured in the indicated mice; $n = 3$ mice/group.

B Schematic of the TRF-promoter-PPRE.

C A 2,000-base pair fragment of the promoter of mouse TRF was inserted into the pGL3 promoter vector to generate the pGL3 promoter constructs. These reporter constructs were transfected into Hep1-6 cells. The indicated PPARα ligands were added to cell cultures 24 h before the reporter gene assay. Data were calculated as the fold induction with respect to the empty vector (pGL3 promoter luciferase vector). $n = 3$ biological replicates.

D Chromatin immunoprecipitation assays were performed on soluble formaldehyde–crosslinked chromatin isolated from untreated and GW7647-treated WT or PPARα$^{-/-}$ livers with polyclonal anti-PPARα antibodies (anti-PPARα) or control IgG. The final DNA extraction was polymerase chain reaction-amplified with a primer pair that covered the sequence in the promoter of Gpx4.

Data information: mRNA levels were normalized to 36B4 and are expressed relative to the mean value of the WT group; Data are presented as means ± SD. *$P < 0.05$, determined by ANOVA.

Figure EV4. PPARα deletion increases ferroptosis caused by iron overload.

A Hepatic iron content was measured in 8-week-old WT, PPARα$^{-/-}$ mice that received intraperitoneal injections of dextriferron or saline.
B Serum ALT and AST levels was measured in 8-week-old WT, PPARα$^{-/-}$ mice that received intraperitoneal injections of dextriferron or saline.
C Liver sections were obtained from the indicated mice and stained with H&E. All scale bars are 50 μm. $n = 3$ biological replicates.
D Hepatic MDA content was measured in 8-week-old WT, PPARα$^{-/-}$ mice that received intraperitoneal injections of dextriferron or saline.
E Measurement of intracellular ROS levels by fluorescent probe DCFH-DA, and the fluorescence intensity of ROS was calculated. All scale bars are 20 μm. $n = 3$ biological replicates.
F Hepatic Gpx4 mRNA levels were measured in the indicated mice.
G Hepatic Gpx4 mRNA levels were measured in the mice that were fed a HID with or without Gpx4-AAV treatment.

Data information: In (A–G), $n = 3$–5 mice/group. mRNA levels were normalized to 36B4 and are expressed relative to the mean value of the WT group; Data are presented as means ± SD. *$P < 0.05$, **$P < 0.01$, determined by ANOVA.

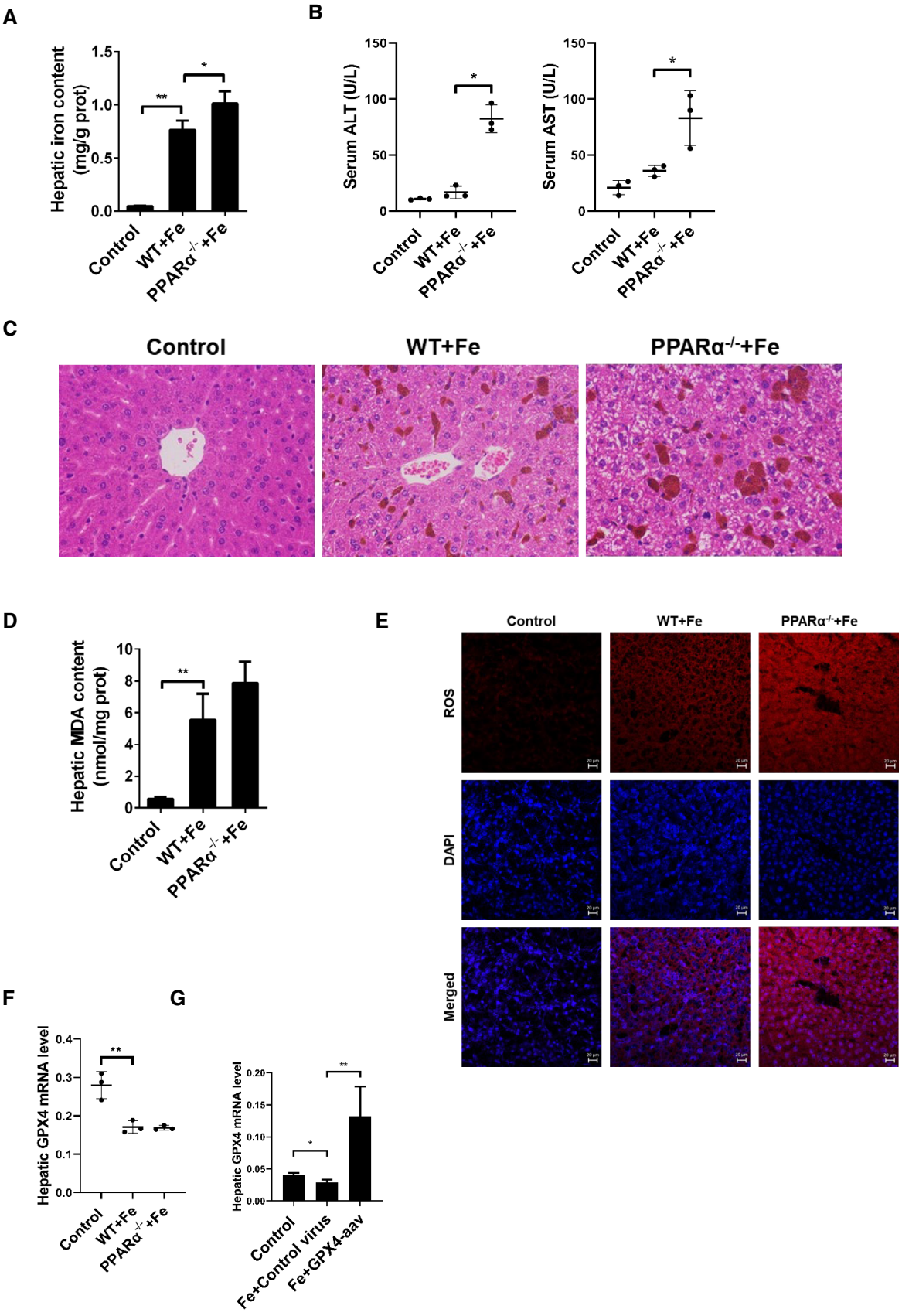
A
Hepatic iron content (mg/g prot)
1.5
1.0
0.5
0.0
**
*
Control
WT+Fe
PPARα-/-+Fe
B
Serum ALT (U/L)
150
100
50
0
*
Control
WT+Fe
PPARα-/-+Fe
Serum AST (U/L)
150
100
50
0
*
Control
WT+Fe
PPARα-/-+Fe
C
Control
WT+Fe
PPARα-/-+Fe
D
Hepatic MDA content (nmol/mg prot)
10
8
6
4
2
0
**
Control
WT+Fe
PPARα-/-+Fe
E
Control
WT+Fe
PPARα-/-+Fe
ROS
DAPI
Merged
F
Hepatic GPX4 mRNA level
0.4
0.3
0.2
0.1
0.0
**
Control
WT+Fe
PPARα-/-+Fe
G
Hepatic GPX4 mRNA level
0.20
0.15
0.10
0.05
0.00
*
**
Control
Fe+Control virus
Fe+GPX4-aav

Figure EV4.