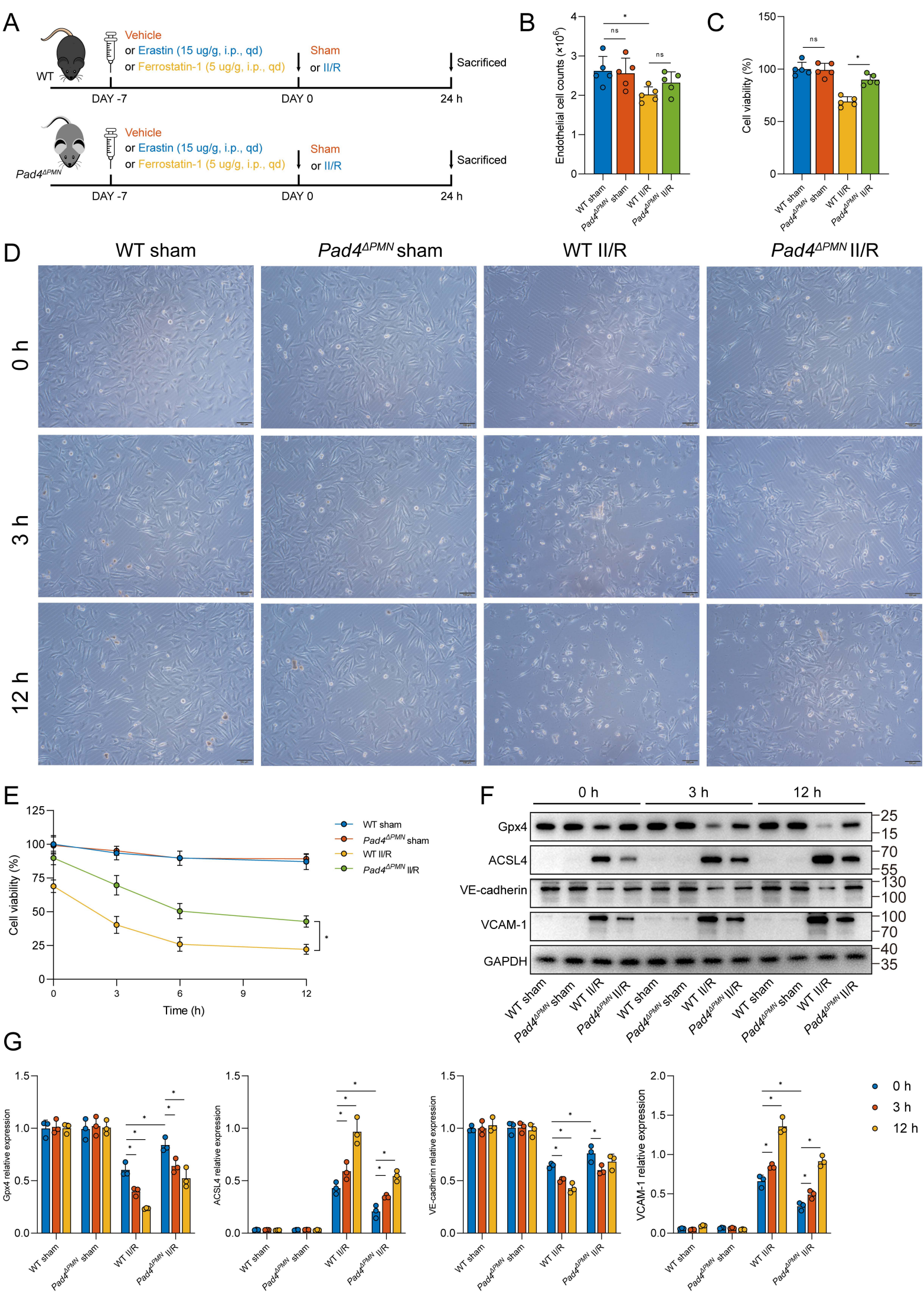
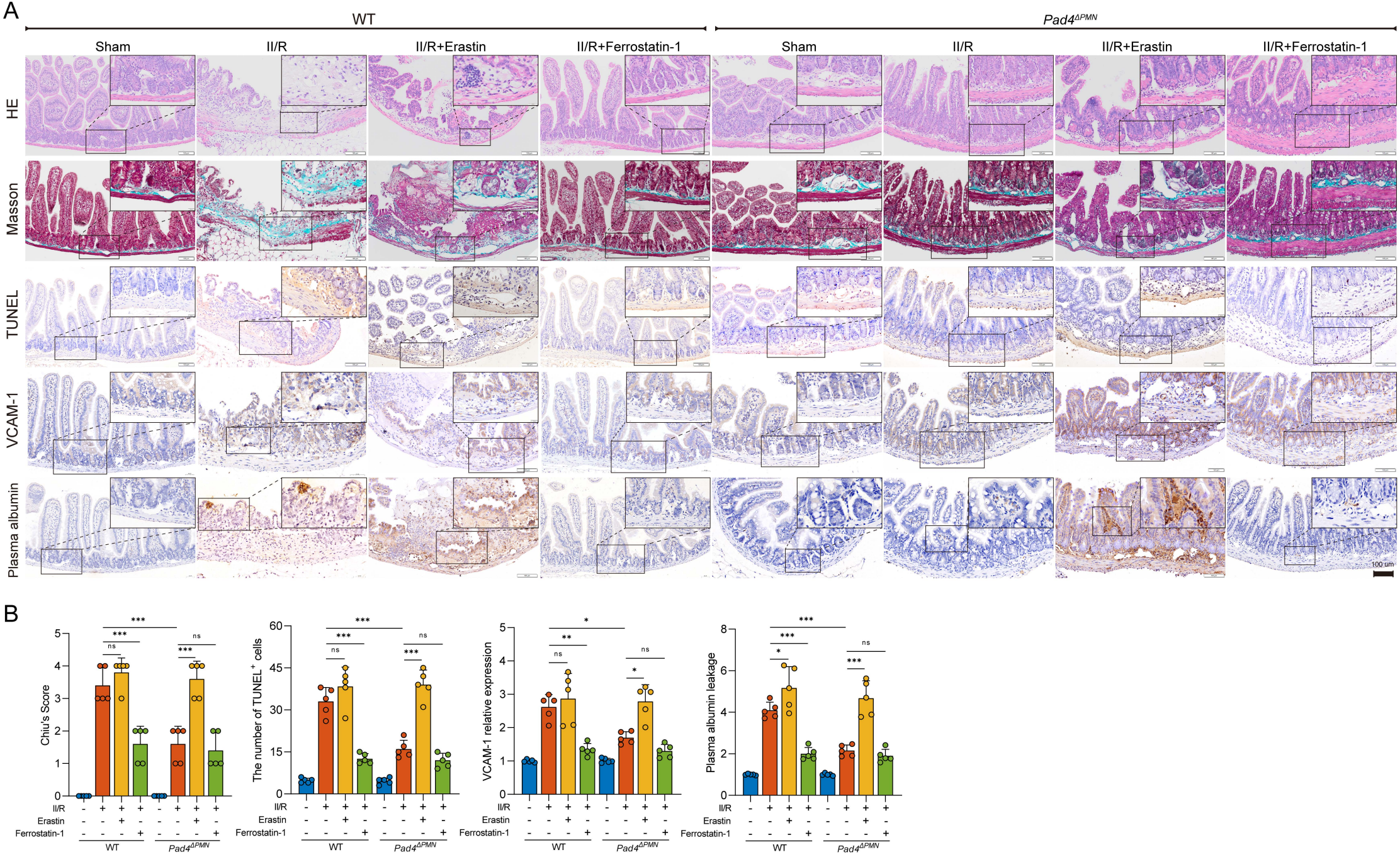

A
WT
Vehicle
or Erastin (15 ug/g, i.p., qd)
or Ferrostatin-1 (5 ug/g, i.p., qd)
Sham
or II/R
Sacrificed
DAY -7
DAY 0
24 h
Pad4ΔPMN
B
Endothelial cell counts (×10^6)
C
Cell viability (%)
WT sham
Pad4ΔPMN sham
WT II/R
Pad4ΔPMN II/R
D
0 h
3 h
12 h
E
Cell viability (%)
Time (h)
F
Gpx4
ACSL4
VE-cadherin
VCAM-1
GAPDH
G
Gpx4 relative expression
ACSL4 relative expression
VE-cadherin relative expression
VCAM-1 relative expression

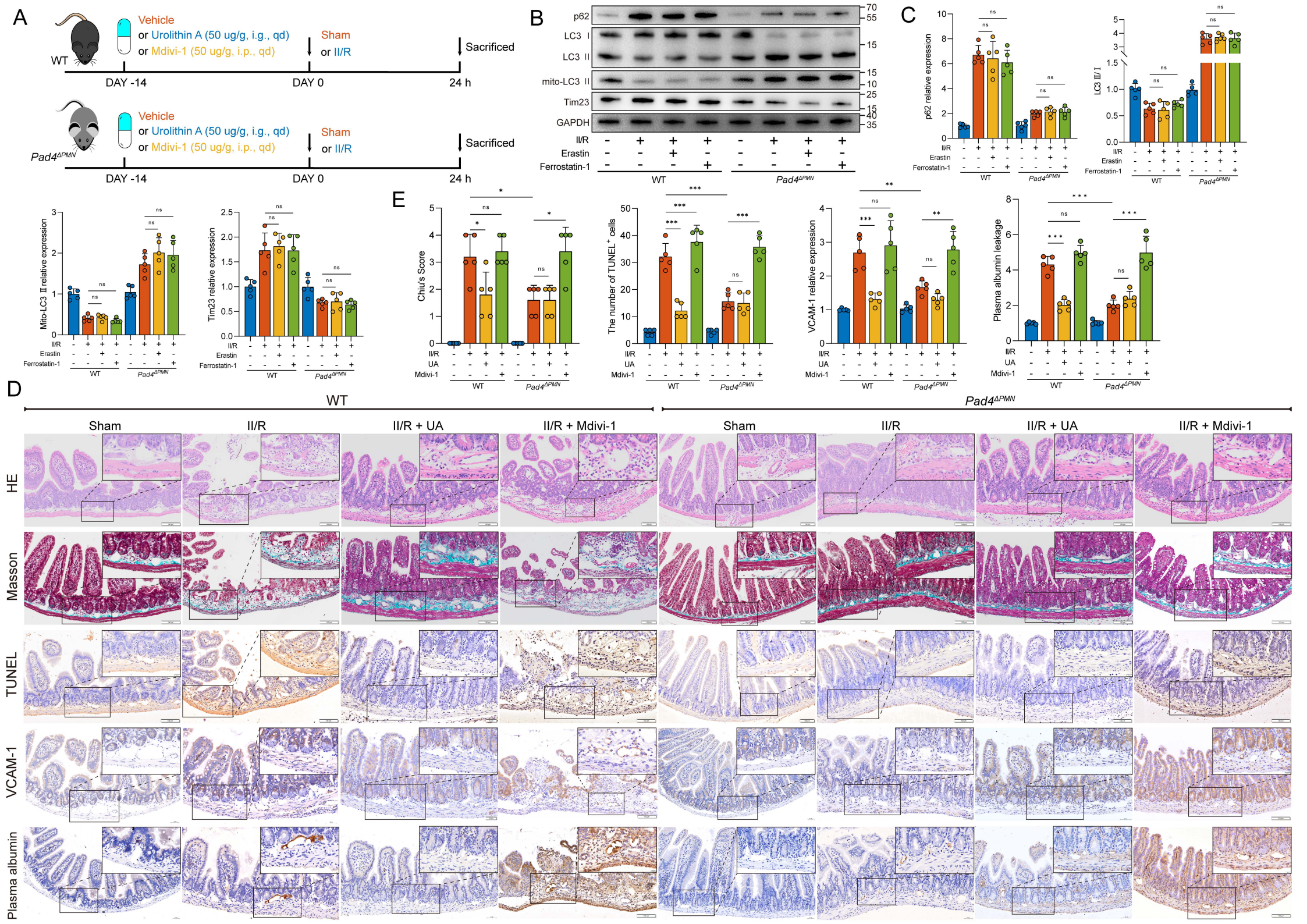
Supplementary Figure 1

A. Schematic diagram of drug administration to WT and *Pad4*$^{\Delta PMN}$ mice. The ferroptosis activator erastin, the ferroptosis inhibitor ferrostatin-1 and the vehicle were administered intraperitoneally for 7 days before II/R. **B.** Endothelial cell count was obtained by isolating them from a total of 1×10^8 cells derived from the intestines. **C.** Cell viability of the recently isolated endothelial cells was assessed using the CCK8 assay kit. **D**. The morphology of extracted endothelial cells from the intestinal microvasculature was observed using an optical microscope at various incubation times (0 h, 3h, 12h) in *vitro*. **E**. The cell viability-time curves were estimated using CCK8 assay. **F-G**. Cellular ferroptosis levels (Gpx4 and ACSL4) and endothelial cell damage markers (VE-cadherin and VCAM-1) were detected using Western blotting. Data are shown as the means ± SD, ns, not significant, *$p < 0.05$.


A
WT
Pad4ΔPMN
Sham
II/R
II/R+Erastin
II/R+Ferrostatin-1
HE
Masson
TUNEL
VCAM-1
Plasma albumin
100 um
B
Chiu's Score
The number of TUNEL+ cells
VCAM-1 relative expression
Plasma albumin leakage
II/R
Erastin
Ferrostatin-1
WT
Pad4ΔPMN
ns

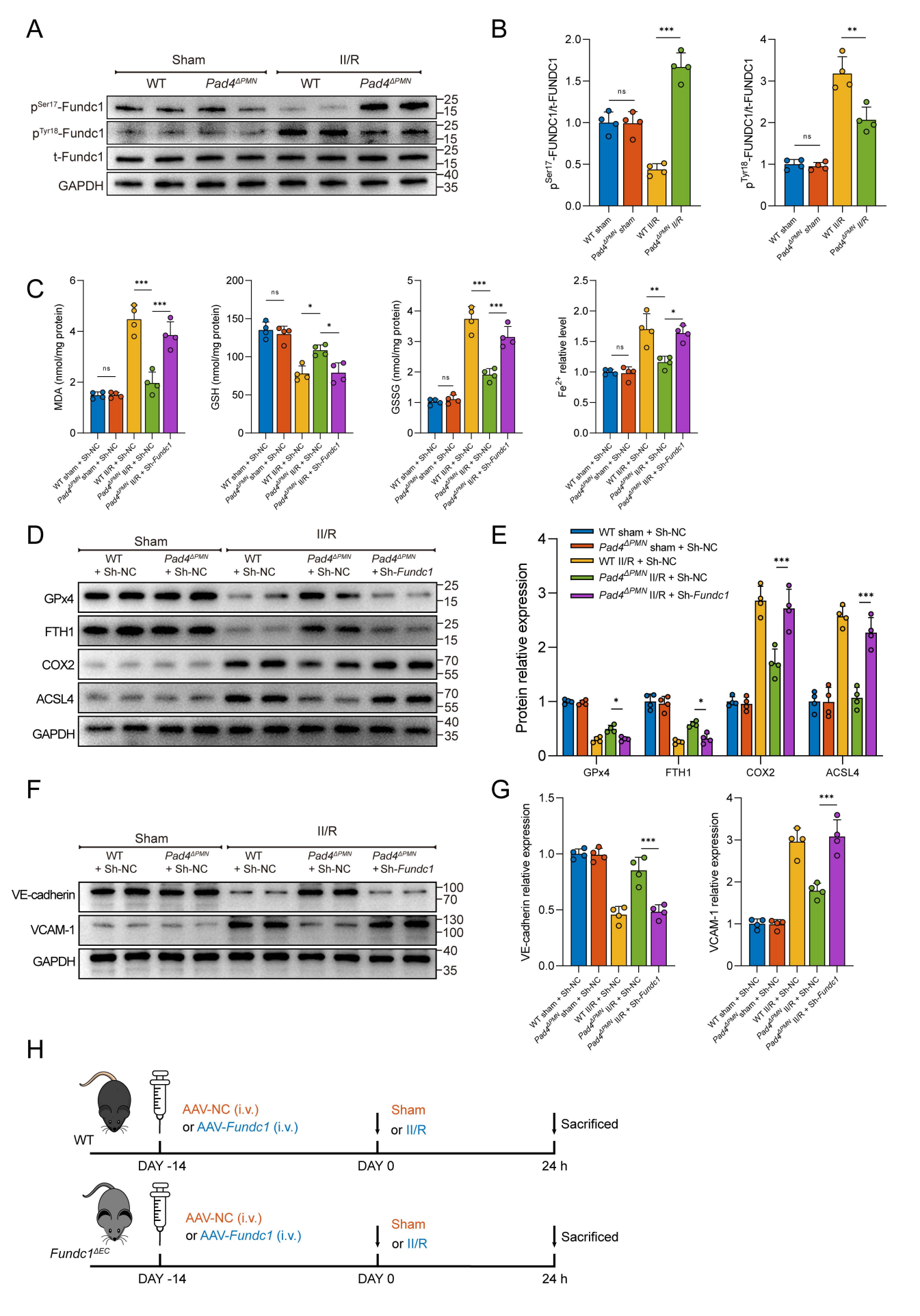
Supplementary Figure 2

A-B. Intestinal tissue from mice was used for pathological analysis and immunohistochemical staining. The Chiu score system was used to assess HE- and Masson's trichrome-stained tissue samples from the intestine. To detect apoptosis of intestinal microvascular endothelial cells, TUNEL staining was performed, and the proportion of TUNEL-positive cells was determined. The quantification of VCAM-1 and albumin through immunohistochemical staining of intestinal sections was conducted to evaluate microvascular permeability and function. Data correspond to the means ± SD, ns, not significant, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.


A
WT
Vehicle
or Urolithin A (50 ug/g, i.g., qd)
or Mdivi-1 (50 ug/g, i.p., qd)
Sham
or II/R
Sacrificed
DAY -14
DAY 0
24 h
Pad4ΔPMN
B
p62
LC3 I
LC3 II
mito-LC3 II
Tim23
GAPDH
II/R
Erastin
Ferrostatin-1
C
p62 relative expression
LC3 II/ I
E
Chiu's Score
The number of TUNEL+ cells
VCAM-1 relative expression
Plasma albumin leakage
UA
Mdivi-1
D
Sham
II/R
II/R + UA
II/R + Mdivi-1
HE
Masson
TUNEL
VCAM-1
Plasma albumin

Supplementary Figure 3

A. Schematic diagram depicting the drug administration process in both WT and *Pad4*$^{\Delta PMN}$ mice. **B-C**. Effects of ferroptosis regulation on the degree of mitophagy were determined by western blot analysis. **D-E**. Pathological staining and immunohistochemistry were conducted to evaluate the impact of a mitophagy activator (UA) and inhibitor (Mdivi-1) on intestinal microvascular injury in WT and *Pad4*$^{\Delta PMN}$ mice. UA, urolithin A; Mdivi-1, mitochondrial division inhibitor 1. Data are shown as the means ± SD, ns, not significant, *p < 0.05, **p < 0.01, ***p < 0.001.


A
Sham
II/R
WT
Pad4ΔPMN
WT
Pad4ΔPMN
pSer17-Fundc1
pTyr18-Fundc1
t-Fundc1
GAPDH
B
pSer17-FUNDC1/t-FUNDC1
pTyr18-FUNDC1/t-FUNDC1
WT sham
Pad4ΔPMN sham
WT II/R
Pad4ΔPMN II/R
C
MDA (nmol/mg protein)
GSH (nmol/mg protein)
GSSG (nmol/mg protein)
Fe2+ relative level
WT sham + Sh-NC
Pad4ΔPMN sham + Sh-NC
WT II/R + Sh-NC
Pad4ΔPMN II/R + Sh-NC
Pad4ΔPMN II/R + Sh-Fundc1
D
Sham
II/R
WT + Sh-NC
Pad4ΔPMN + Sh-NC
Pad4ΔPMN + Sh-Fundc1
GPx4
FTH1
COX2
ACSL4
GAPDH
E
Protein relative expression
WT sham + Sh-NC
Pad4ΔPMN sham + Sh-NC
WT II/R + Sh-NC
Pad4ΔPMN II/R + Sh-NC
Pad4ΔPMN II/R + Sh-Fundc1
GPx4
FTH1
COX2
ACSL4
F
Sham
II/R
WT + Sh-NC
Pad4ΔPMN + Sh-NC
Pad4ΔPMN + Sh-Fundc1
VE-cadherin
VCAM-1
GAPDH
G
VE-cadherin relative expression
VCAM-1 relative expression
H
WT
AAV-NC (i.v.)
or AAV-Fundc1 (i.v.)
Sham
or II/R
Sacrificed
DAY -14
DAY 0
24 h
Fundc1ΔEC
AAV-NC (i.v.)
or AAV-Fundc1 (i.v.)
Sham
or II/R
Sacrificed
DAY -14
DAY 0
24 h

Supplementary Figure 4

A-B. Western blotting was performed to determine p^{Ser17}-Fundc1 and p^{Tyr18}-Fundc1 protein levels in WT and *Pad4*$^{\Delta PMN}$ mice. **C**. Intracellular MDA, GSH, GSSH and Fe2+ levels in isolated intestinal endothelial cells were assessed by ELISAs. **D-E**. Levels of ferroptosis-related proteins in the intestinal endothelium of WT and *Pad4*$^{\Delta PMN}$ mice pretreated with or without a *Fundc1* shRNA were determined using western blotting. **F-G**. Western blotting was performed to examine the expression of the intestinal microvascular damage markers VCAM-1 and VE-cadherin. The grayscale values of the bands were determined using ImageJ. **H**. Schematic diagram of tail vein injection of AAV9 containing *Fundc1* or AAV9 control in WT and *Fundc1*$^{\Delta EC}$ mice. AAV, adeno-associated virus. Data are presented as the means ± SD, ns, not significant, *p < 0.05, **p < 0.01, ***p < 0.001.