The legend of supplemental Movie

Supplemental Movie 1. Additional data demonstrating echocardiography of WT and *Bach1*$^{-/-}$ mice before and after operation. (*A-D*) Representative movie of echocardiography of WT mice 1 week before operation (*A*) and 2 weeks after operation (*B*) and *Bach1*$^{-/-}$ mice 1 week before operation (*C*) and 2 weeks after operation (*D*). These movies represent long-axis views of cardiac infarction area. The more detailed information of supplementary movie 1 was put in Additional explanation figure for supplemental movie.

Supplemental Table 1. The list of MRM for targeted Compounds

Negative ion mode

Compound Name	Precursor ion (*m/z*)	Product ion (*m/z*)	Cone voltage (V)	Collision energy (eV)
GSH	306.12	143.02	32	16
GSH-$^{13}C_2$, ^{15}N	309.07	146.02	24	20
GSSG	611.25	272.08	2	28

Supplemental Table 2. siRNA

siRNA	Sequence
si*Gclm*#1	5'-GAGGAGCUUCGGGACUGUAUCCAAA-3'
si*Gclm*#3	5'-GCUAAACAAUUUGACAUACAGCUAC-3'
si*Slc7a11*#1	5'-UGGCAGUCGCAGGACUGAUUUAUCU-3'
si*Slc7a11*#2	5'-ACUGAAGAAGUAGACAACCCUGAAA-3'
si*Gclc*#2	5'-CCCGCUUCGGUACUCUAACAAGAAA-3'
si*Gclc*#3	5'-GCUUCUCAGCCAGACCAUAUCUACA-3'
si*Hmox1*#1	5'-GGCAGUGGGAAUUUAUGCCAUGUAA-3'
si*Hmox1*#3	5'-CAGCUCUAUCGUGCUCGAAUGAACA-3'

Supplemental Table 3. qPCR primers

Gene name	Forward primer	Reverse primer
Actb	CGTTGACATCCGTAAAGACCTC	AGCCACCGATCCACACAGA
Bach1	GCCCGTATGCTTGTGTGATT	CGTGAGAGCGAAATTATCCG
Fth1	CGAGGTGGCCGAATCTTCC	TGCAGTTCCAGTAGTGACTGAT
Ftl1	ATGAATGGGGTAAAACCCAGGA	GAGTGAGGCGCTCAAAGAGAT
Fxn	CAAGCAGACCCCAAACAAGC	CAGTTCTTCCCGGTCCAGTC
Gclc	GCATCCTCCAGTTCCTGCAC	GGTCGGATGGTTGGGGTTTG
Gclm	AGTGGGCACAGGTAAAACCC	CTGGGCTTCAATGTCAGGGA
Gpx4	CTAGTCGATCTGCATGCCCG	CAAACTGGTTGCAGGGGAAG
Hmox1	GGGTGACAGAAGAGGCTAAG	GTGTCTGGGATGAGCTAGTG
Mfn2	CGCCAGTTTGTGGAATACGC	TCGGGTGATGTCAACTTGCT
Mt1	CCTCTAAGCGTCACCACGAC	AGTTGGGGTCCATTCCGAGA
Slc40a1	AGCAGCTAAAGCTACCAGCA	AAAGTGCCACATCCGATCCC
Slc7a11	GGGCCCATAATCAACGGAGAG	CACGGATCTCCAGGGCTTCT
Tfrc	GGGTACTCCCTTGTAGCAGC	TGGTTCCCCACCAAACAAGT

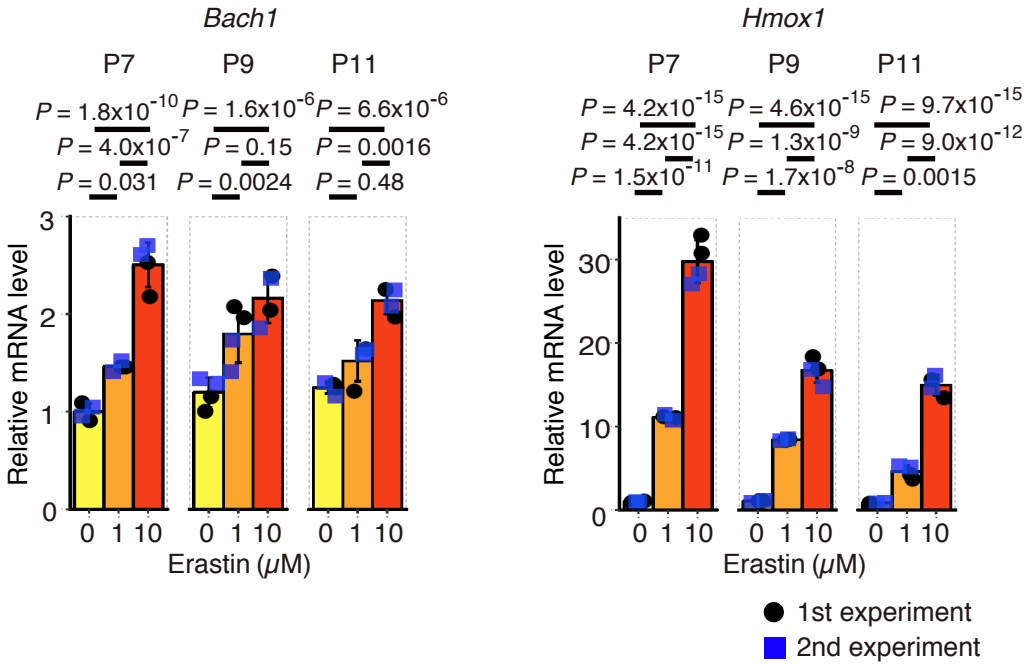

Figure S1. The transcription of *Bach1* and *Hmox1* increases during ferroptosis. WT MEFs (7th, 9th, and 11th passage : P7, P9, and P11) were exposed to erastin for 10 hrs. qPCR analysis for *Bach1* and *Hmox1* mRNA relative to *Actb* mRNA. Error bars represent standard deviation. *P*-value by Tukey's test after two-way ANOVA.

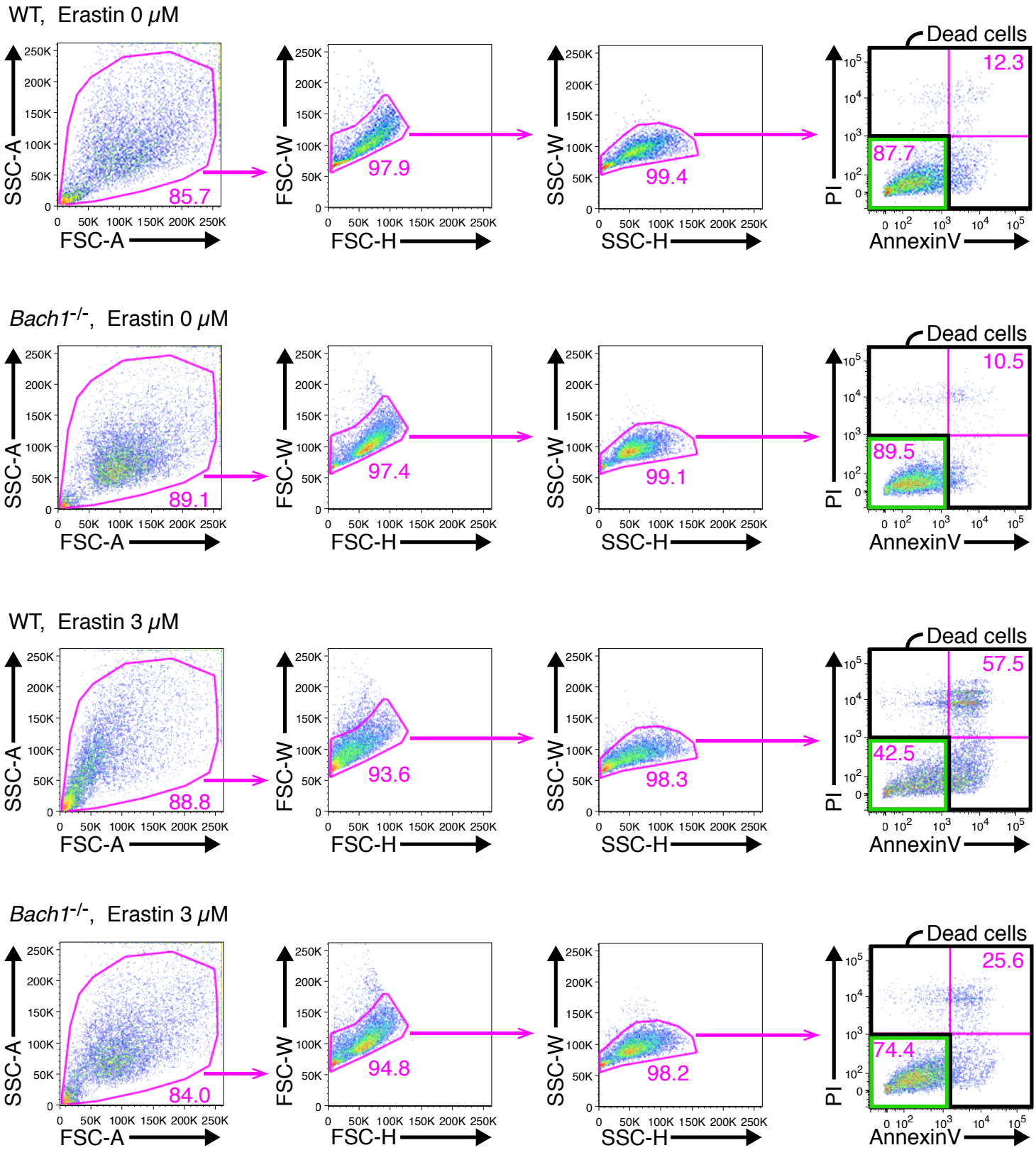

Figure S2. Additional data demonstrating flow cytometry gating of dead cells in WT and *Bach1*$^{-/-}$ MEFs exposed to erastin. WT and *Bach1*$^{-/-}$ MEFs (11th passage: P11) exposed to erastin for 24 hrs (Fig. 2*B*). Representative flow cytometry images showing the strategy that was implemented for the sorting of dead cells. FSC-A (forward scatter area) and SSC-A (side scatter area) gating was used to identify cells of interest (including dead cells). FSC-W (FSC width) and FSC-H (FSC height) gating and SSC-W (SSC width) and SSC-H (SSC height) gating were used to exclude doublet cells from analysis. Propidium iodide (PI) positive or Annexin V positive cells were judged as dead cells. The similar strategy was implemented in Fig. 2*E*, Fig. 4*C* and *D*, Fig. S3*B*, Fig. S7*A*-*D*, Fig. S9*B*, and Fig. S10*E*.

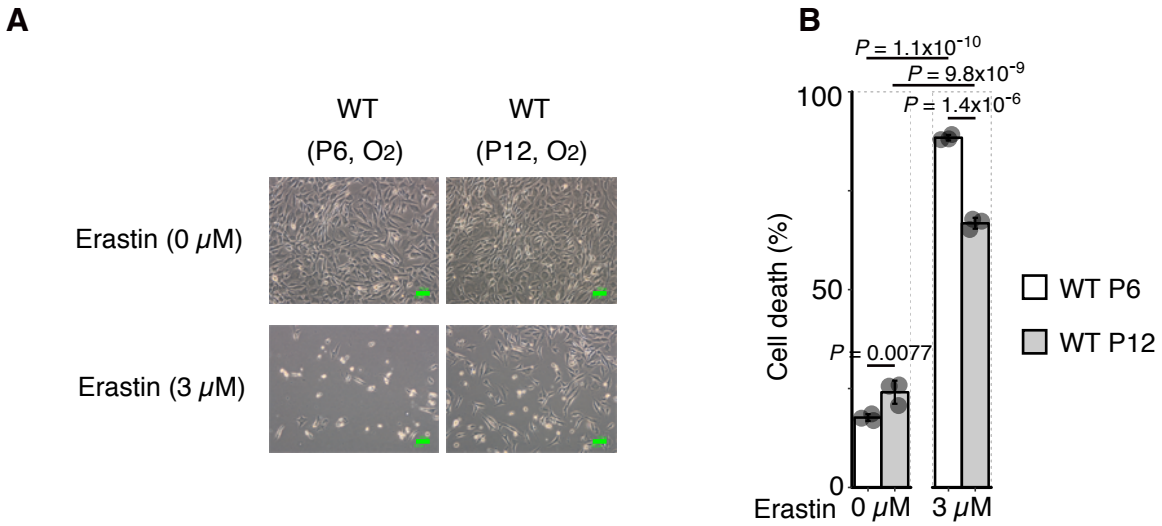

Figure S3. Senescent cells get resistance to ferroptosis. (*A, B*) Optical microscope image (*A*) and Quantification of cell death by flow cytometer (*B*) of WT MEFs (6th passage: P6, 12th passage: P12) exposed to erastin for 30 hrs. Scale bars in (*A*) represent 100 μm. Error bars of (*B*) represent standard deviation. *P*-value by Tukey's test after two-way ANOVA.

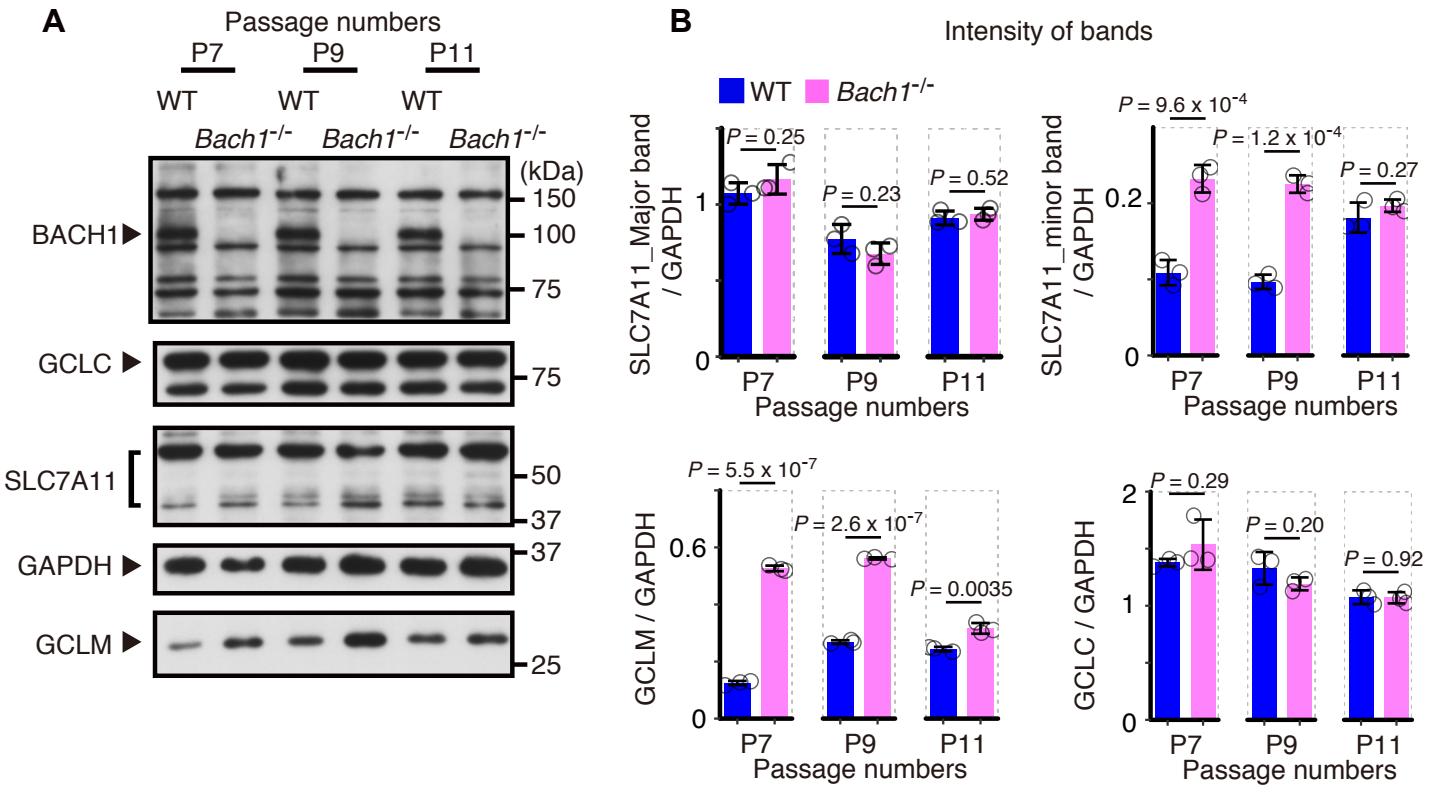

Figure S4. Additional data demonstrating BACH1 decreases expression of GCLM protein. (*A, B*) Western blotting for BACH1, SLC7A11, GCLM, GCLC, and GAPDH of WT and *Bach1*$^{-/-}$ MEFs (P7, P9, P11). Representative image (*A*) and the intensity of bands (*B*). Error bars of (*B*) represent standard deviation. *P*-value by *t*-test.

Figure S5

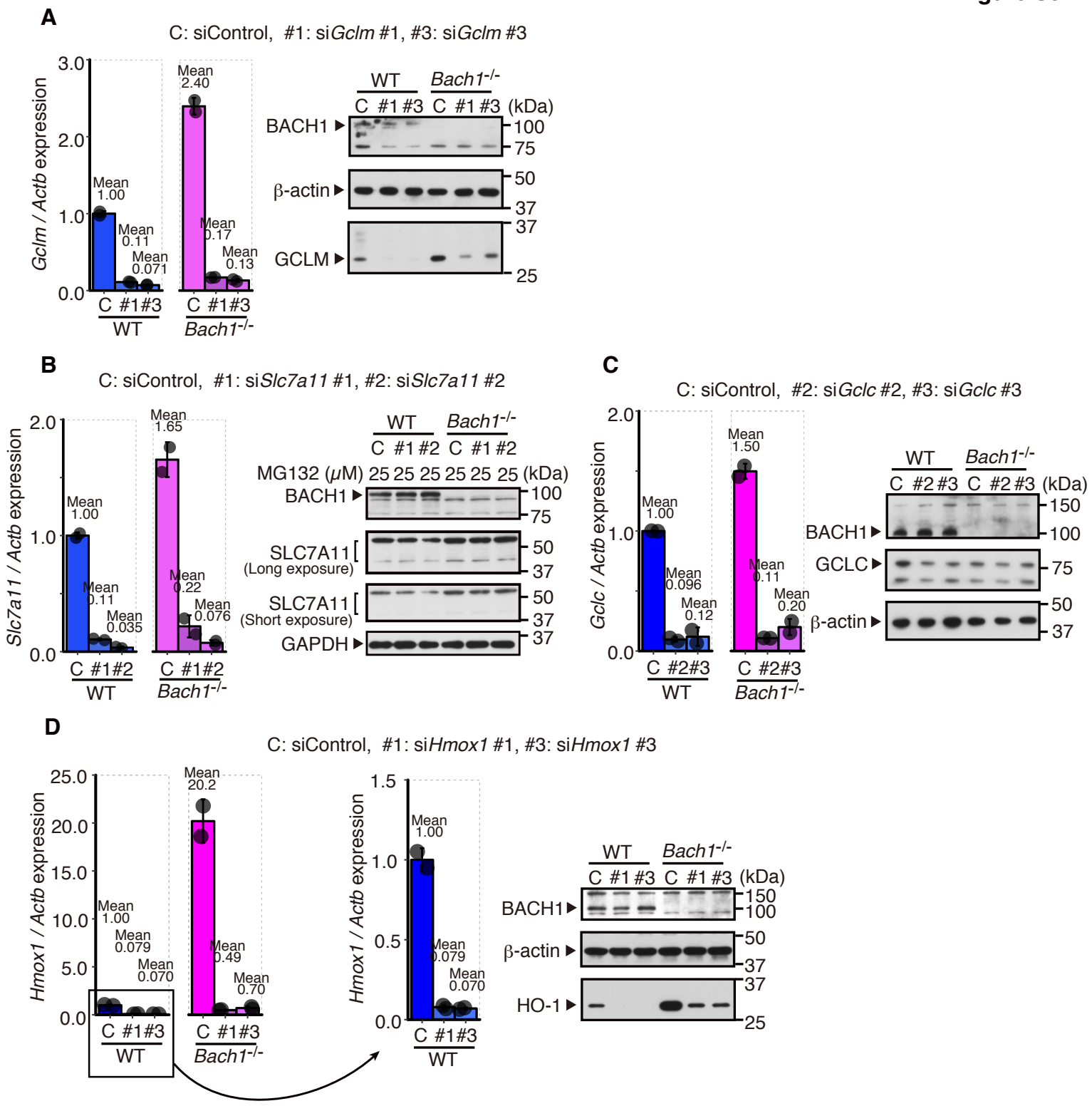

Figure S5. Knockdown of *Gclm*, *Slc7a11*, *Gclc*, and *Hmox1* in Fig. S6 and S7. (*A-D*) siRNA was transfected to WT and *Bach1*$^{-/-}$ MEFs (5th or 6th passage). qPCR analysis for *Gclm*, *Slc7a11*, *Gclc*, and *Hmox1* mRNA relative to *Actb* mRNA (*A-D*, left part). Western blotting for BACH1, GCLM, SLC7A11, GCLC, HO-1, β-actin, and GAPDH of MEFs (*A-D*, right part). Error bars of (*A-D*) represent standard deviation.

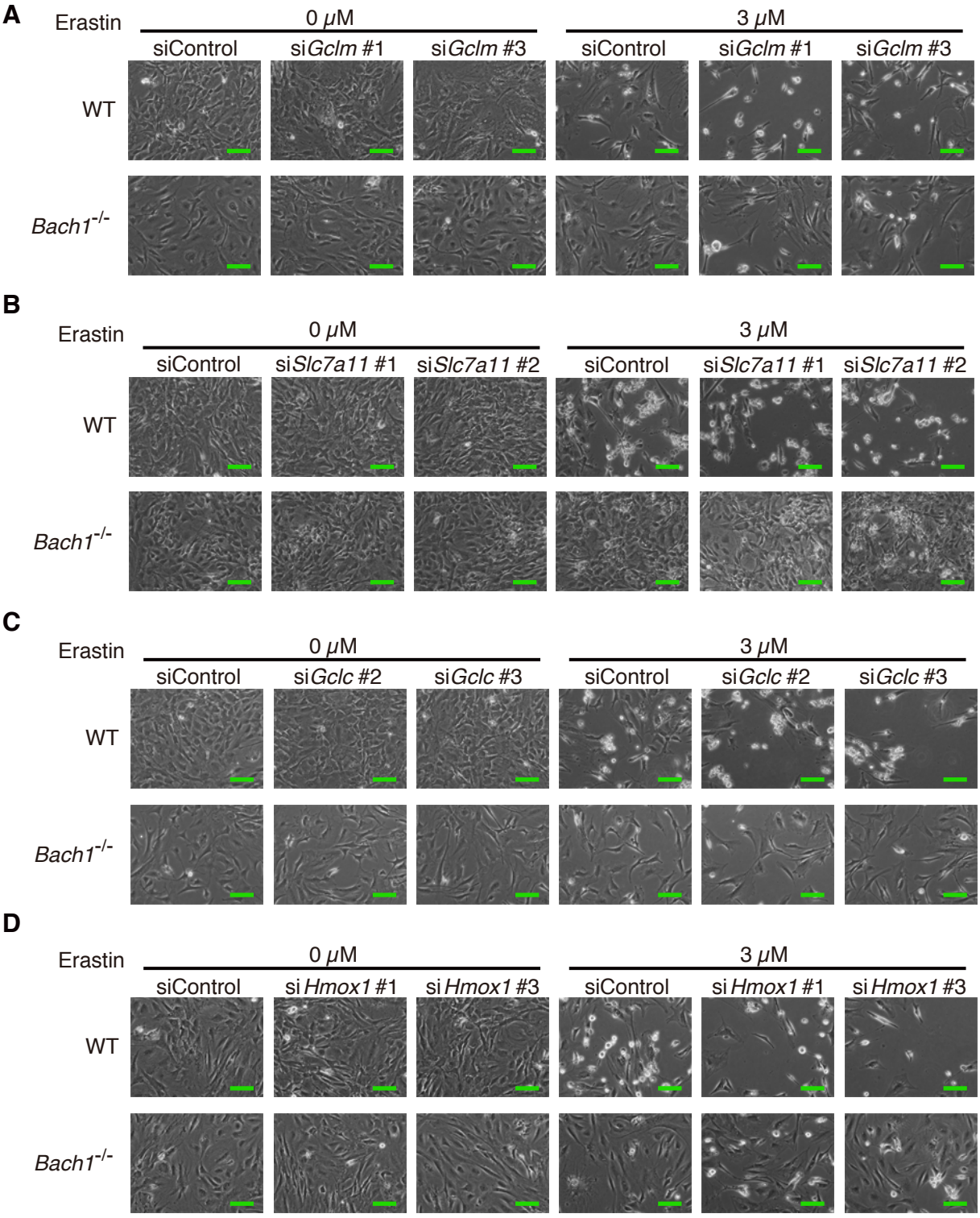

Figure S6. *Gclm*, *Slc7a11*, *Gclc*, and *Hmox1* repress ferroptosis, associated with Fig. S7.

(*A-D*) siRNA was transfected to WT and *Bach1*$^{-/-}$ MEFs (5th or 6th passage). After 24 hrs, MEFs were exposed to erastin for 24 hrs. Optical microscope image. Scale bars represent 100 μm.

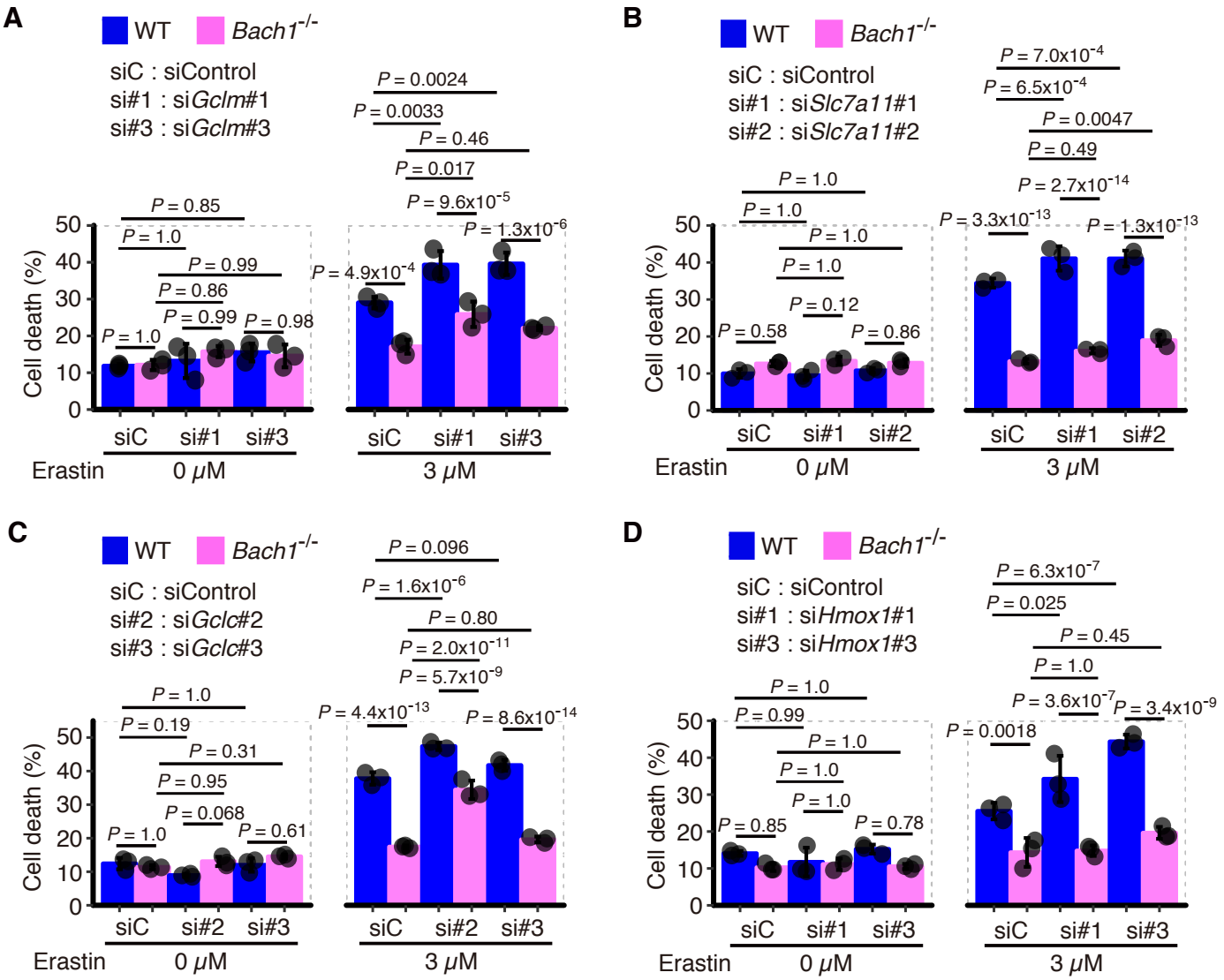

Figure S7. *Gclm*, *Slc7a11*, *Gclc*, and *Hmox1* repress ferroptosis. (*A-D*) siRNA was transfected to WT and *Bach1*$^{-/-}$ MEFs (5th or 6th passage). After 24 hrs, MEFs were exposed to erastin for 24 hrs. Quantification of cell death by flow cytometer. Error bars represent standard deviation. *P*-value by Tukey's test after three-way ANOVA.

Figure S8

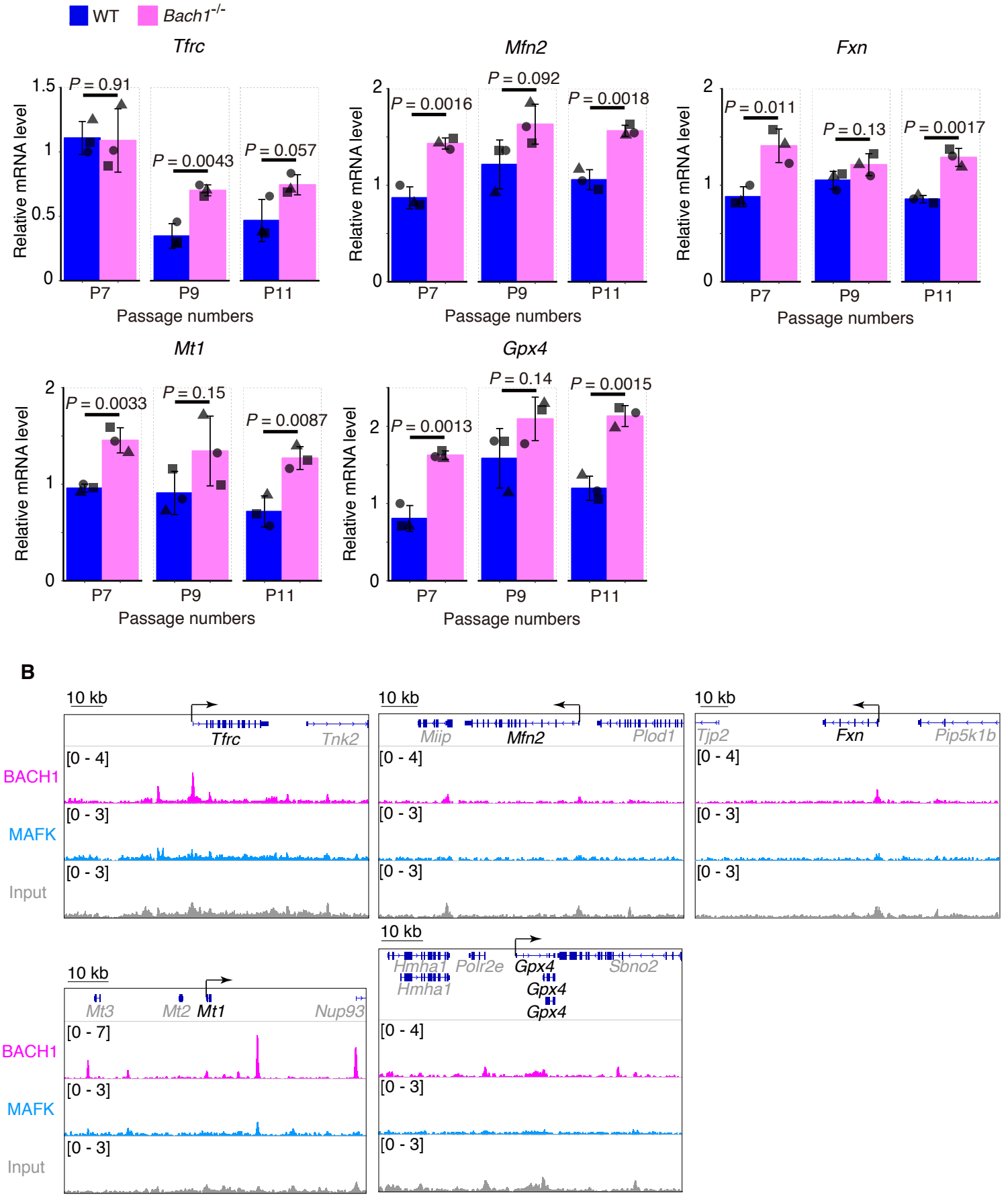

Figure S8. *Tfrc*, *Mfn2*, *Fxn*, *Mt1*, and *Gpx4* were not strongly regulated by BACH1. (*A*) qPCR analysis for *Tfrc*, *Mfn2*, *Fxn*, *Mt1*, and *Gpx4* mRNA relative to *Actb* mRNA in WT and *Bach1*$^{-/-}$ MEFs (7th, 9th, and 11th passage : P7, P9, and P11). (*B*) ChIP-seq analysis of the binding of BACH1, MAFK for gene regions of *Tfrc*, *Mfn2*, *Fxn*, *Mt1*, and *Gpx4* in M1 cells. Error bars of (*A*) represent standard deviation. *P*-value of (*A*) by *t*-test.

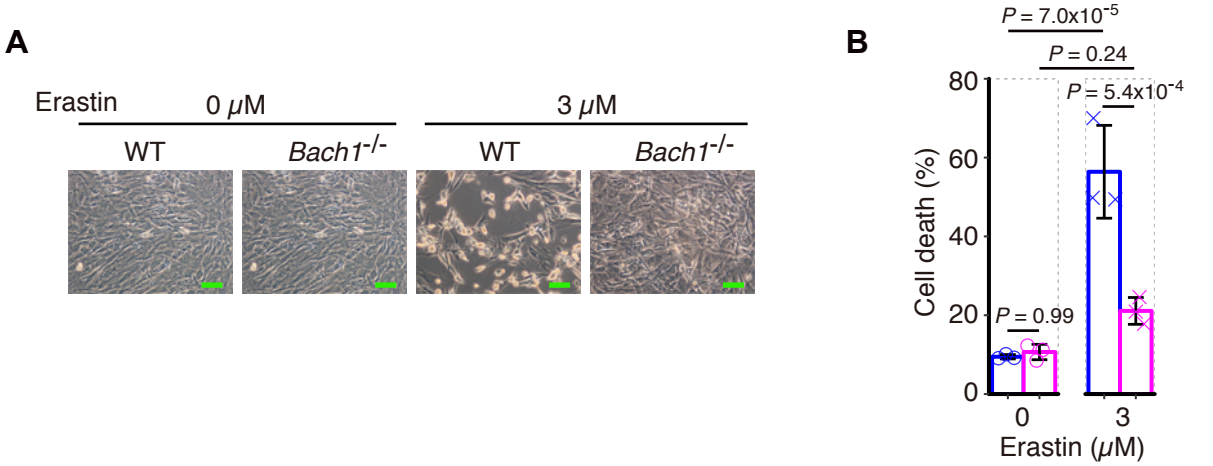

Figure S9. Additional data associated with Fig. 4. (*A, B*) In Fig. 4*C* and *D*, WT and *Bach1*$^{-/-}$ MEFs (8th passage: P8) exposed to erastin for 24 hrs. Optical microscope image (A) and Quantification of cell death by flow cytometer (*B*). Scale bars in (*A*) represent 100 μm. Error bars of (*B*) represent standard deviation. *P*-value of (*B*) by Tukey's test after two-way ANOVA.

Figure S10

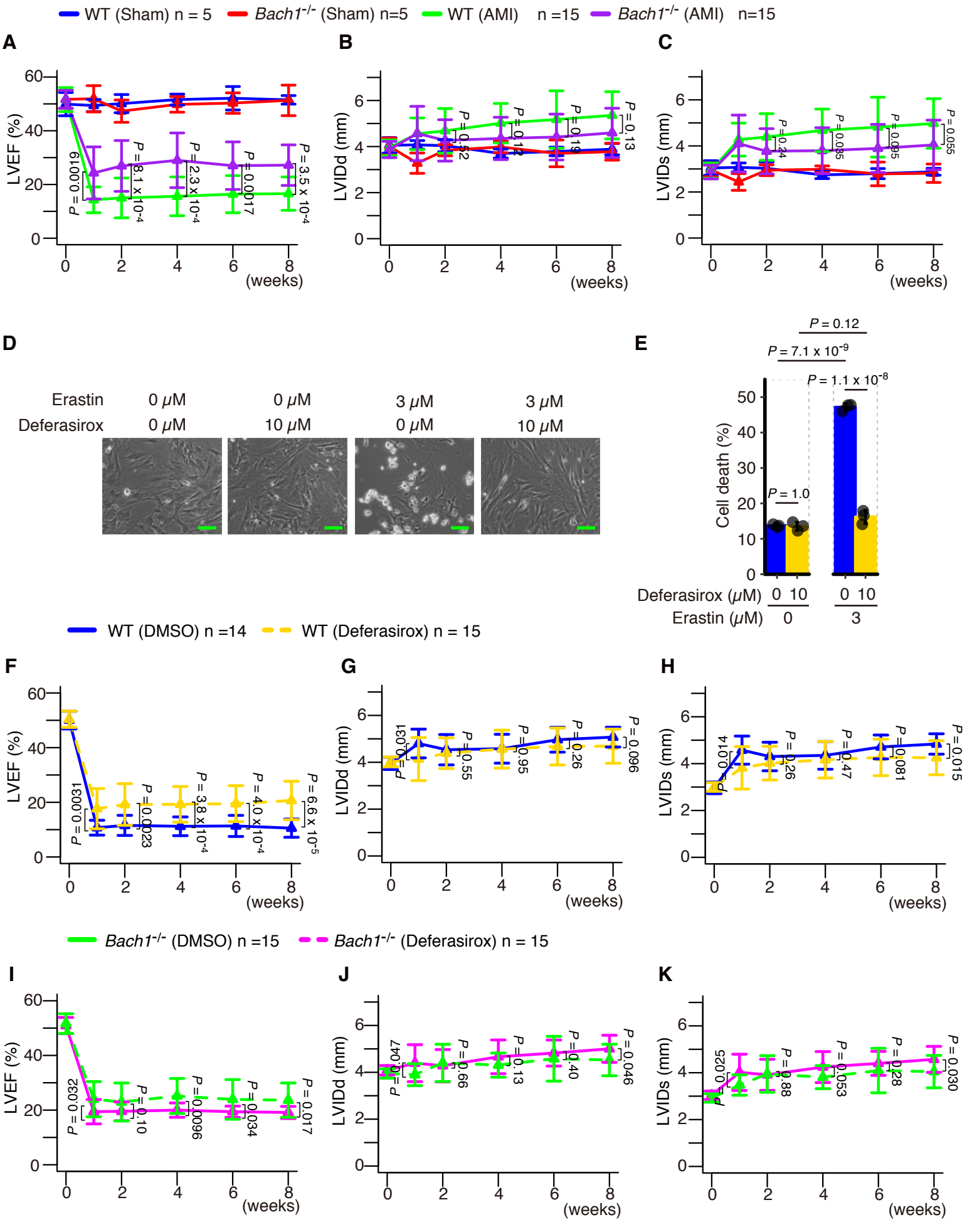
WT (Sham) n = 5
Bach1-/- (Sham) n=5
WT (AMI) n =15
Bach1-/- (AMI) n=15
A
B
C
LVEF (%)
LVIDd (mm)
LVIDs (mm)
(weeks)
P = 0.0019
P = 8.1 x 10-4
P = 2.3 x 10-4
P = 0.0017
P = 3.5 x 10-4
P = 0.52
P = 0.12
P = 0.19
P = 0.13
P = 0.24
P = 0.085
P = 0.095
P = 0.055
D
Erastin 0 μM 0 μM 3 μM 3 μM
Deferasirox 0 μM 10 μM 0 μM 10 μM
E
P = 0.12
P = 7.1 x 10-9
P = 1.1 x 10-8
P = 1.0
Cell death (%)
Deferasirox (μM) 0 10 0 10
Erastin (μM) 0 3
WT (DMSO) n =14
WT (Deferasirox) n = 15
F
G
H
P = 0.0031
P = 0.0023
P = 3.8 x 10-4
P = 4.0 x 10-4
P = 6.6 x 10-5
P = 0.031
P = 0.55
P = 0.95
P = 0.26
P = 0.096
P = 0.014
P = 0.26
P = 0.47
P = 0.081
P = 0.015
Bach1-/- (DMSO) n =15
Bach1-/- (Deferasirox) n = 15
I
J
K
P = 0.032
P = 0.10
P = 0.0096
P = 0.034
P = 0.017
P = 0.047
P = 0.66
P = 0.13
P = 0.40
P = 0.046
P = 0.025
P = 0.88
P = 0.053
P = 0.28
P = 0.030

Figure S10. BACH1 aggravates AMI, that was alleviated by an iron chelator DFX. (*A-C*) Left ventricular ejection fraction (LVEF) (*A*), left ventricular internal dimension in diastole (LVIDd) (*B*), and left ventricular internal dimension in systole (LVIDs) (*C*) on echocardiogram. (*D, E*) Optical microscope image (*D*) and Quantification of cell death by flow cytometer (*E*) of WT and *Bach1*$^{-/-}$ MEFs (10th passage: P10) exposed to erastin for 24 hrs. Scale bars in (*D*) represent 100 μm. (*F-K*) Left ventricular ejection fraction (LVEF) (*F, I*), left ventricular internal dimension in diastole (LVIDd) (*G, J*), and left ventricular internal dimension in systole (LVIDs) (*H, K*) on echocardiogram. Error bars of (*A-C, E-K*) represent standard deviation. *P*-value of (*A-C*) by Tukey-Kramer method after two-way ANOVA. *P*-value of (*E*) by Tukey's test after two-way ANOVA. *P*-value of (*F-K*) by *t*-test.