Expanded View Figures

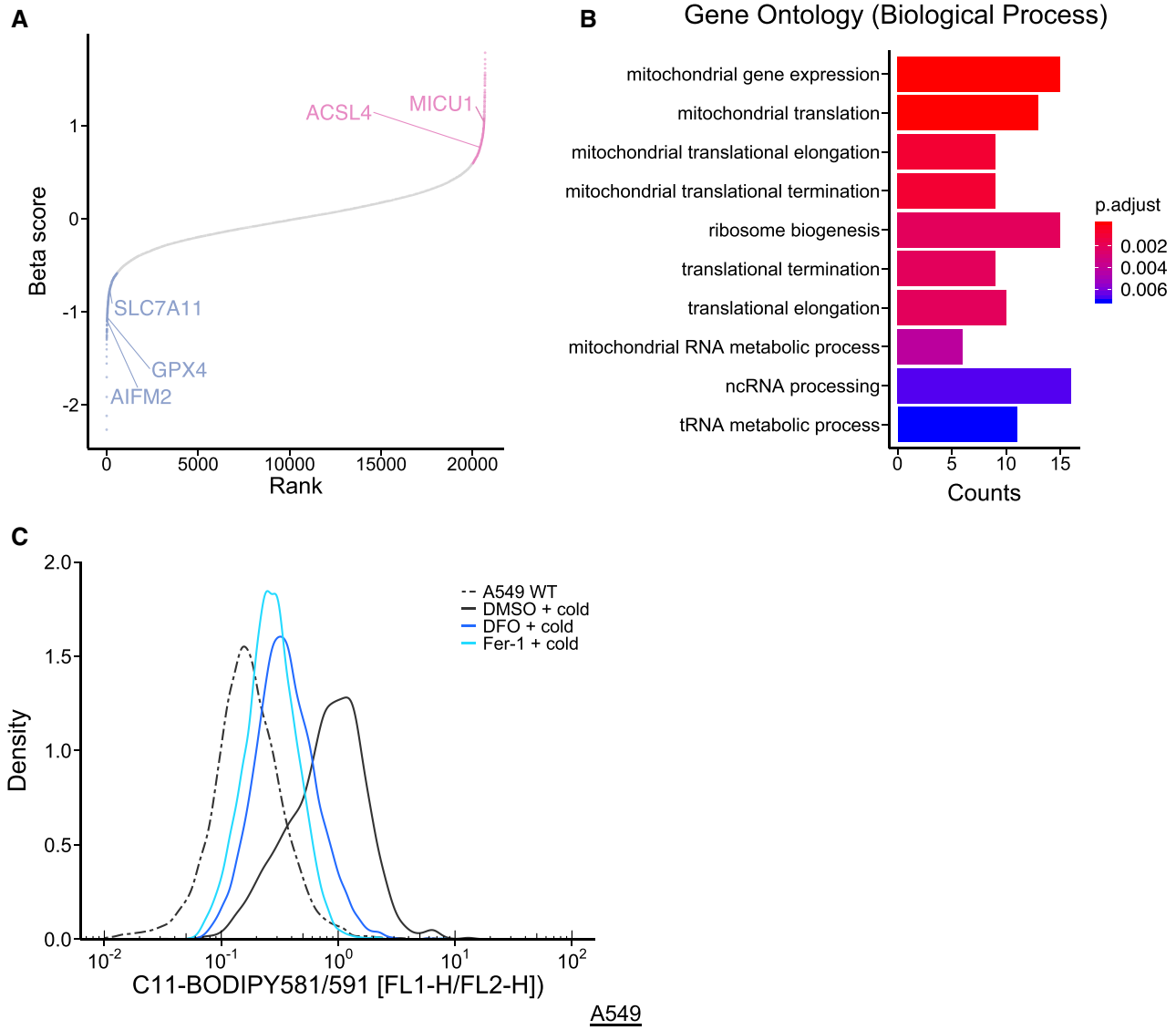

Figure EV1. Analysis of screening, and mitochondria itself is involved in cold stress-induced ferroptosis, related to Fig 1.

A Some genes are described as ferroptosis inducer or suppressors for the adequate evaluation of this screening. A well-known ferroptosis inducer, acyl-CoA synthetase long-chain family member 4 (ACSL4) and ferroptosis suppressors, ferroptosis suppressor 1 (FSP1 also known as AIFM2), glutathione peroxidase 4 (GPX4), and solute carrier family 7 member 11 (SLC7A11), are highlighted after analyzing the count data from CRISPR screening using the MAGeCK program. Red and blue genes are outside of the 2 × SD range and candidates of ferroptosis inducers and suppressors, respectively, which are enriched in MAGeCKFlute packages.

B Results of Gene Ontology (GO) analysis with biological process. After analysis of count data using MAGeCK, positive genes (cut-off of $P < 0.01$, see also Dataset EV1) were analyzed by GO analysis using clusterProfiler packages for R.

C Lipid peroxidation was measured by C11-BODIPY 581/591 after cold stress for 5 h. Representative density plot is presented as reported previously (Hattori *et al*, 2017). Deferoxamine (DFO, 200 μM) and ferrostatin-1 (Fer-1, 1 μM) were pretreated for 30 min before cold stress.

Figure EV2. MCU deficiency does NOT suppress cold stress-induced ferroptosis, related to Figures 1 and 2.

A Immunoblots of endogenous MICU1 or MCU signals after siRNAs transfection in the WT A549 cells.
B LDH release was measured after cold stress for 24 h. Data are presented as mean ± SEM; $n = 4$ (B), biological replicates, $^{**}P < 0.01$, n.s.: not significant, one-way ANOVA followed by Dunnett's test.
C Components of the MCU complex (MCU, MCUR1, MCUb (CCDC109B), MICU1, MICU2, MICU3, and EMRE (SMDT1)) are highlighted in red in the RRA score plot calculated by the MAGeCK program.
D Immunoblots of endogenous MCU signals after Cas9/sgRNA lentivirus infection in the WT A549 cells.
E LDH release was measured after cold stress for 24 h. Data are presented as mean ± SEM; $n = 3$, biological replicates, n.s.: not significant, one-way ANOVA followed by Dunnett's test.
F Immunoblots of endogenous MICU1 or MCU signals after siRNAs transfection in the WT A549 cells and MICU1-KO cells.
G LDH release was measured after cold stress for 24 h. Data are presented as mean ± SEM; $n = 3$, biological replicates, $^{****}P < 0.0001$, $^{*}P < 0.05$, one-way ANOVA followed by Tukey's test.
H Immunoblots of endogenous and overexpressing MCU signals after MCU-HA lentivirus infection in the WT A549 cells.
I LDH release was measured after cold stress for 24 h. Data are presented as mean ± SEM; $n = 3$, biological replicates, n.s.: not significant, unpaired t-test.
J Schematic model for MICU1 and MCU expression and its effect on cell death induced by cold stress.
K LDH release was measured after cold stress for 16 h. Data are presented as mean ± SEM; $n = 3$, biological replicates, n.s.: not significant, one-way ANOVA followed by Dunnett's test.
L MICU2 mRNA was quantified by RT–qPCR. Data are presented as mean ± SEM; $n = 3$, biological replicates, $^{****}P < 0.0001$, one-way ANOVA followed by Dunnett's test.

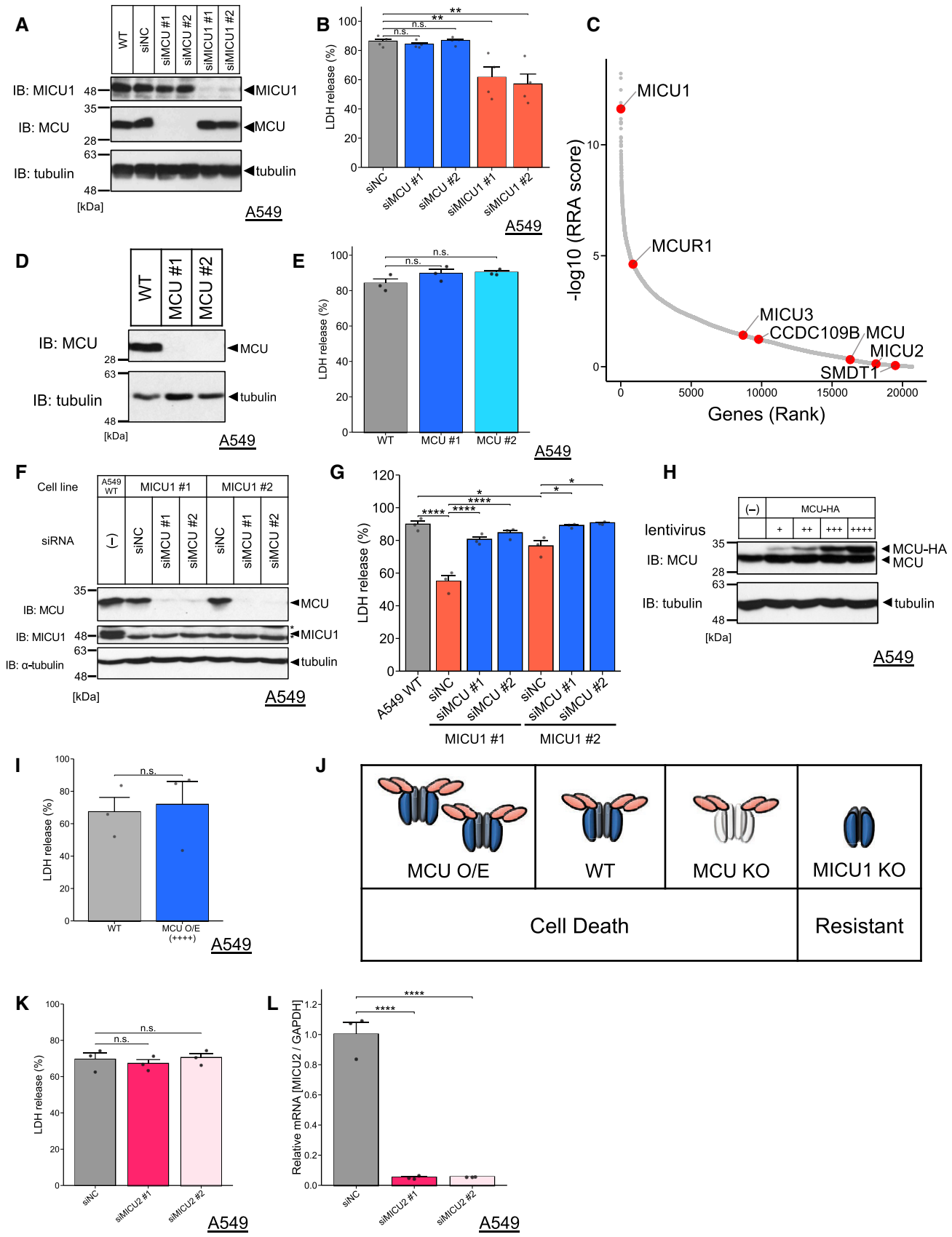

Figure EV2.

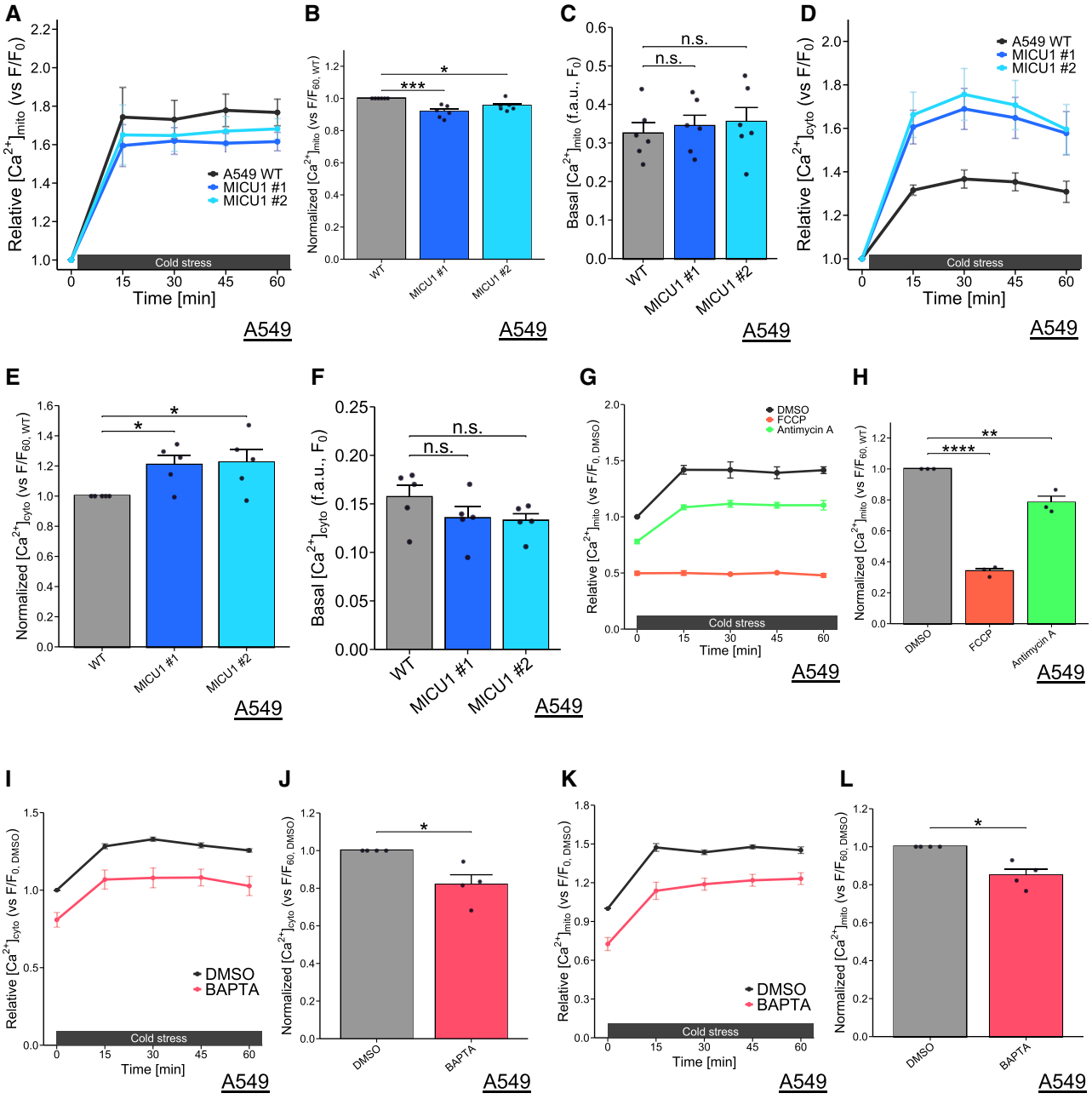

Figure EV3. Cold stress-induced Ca^{2+} increase in matrix and cytosol, related to Figs 1–4.

A-F Mitochondrial Ca^{2+} concentration (A-C) and cytosolic Ca^{2+} concentration (D-F) were monitored by Rhod-2 AM and Cal-520 AM, respectively, at every 15 min. Data (A,B, D and E) were normalized by data of before cold stress. The data before cold stress (C, F) and at 60 min (B, E) are presented as mean ± SEM; $n = 6$ (A-C), 5 (D-F), biological replicates, $^{***}P < 0.001$, $^{*}P < 0.05$, n.s.: not significant, by one-way ANOVA followed by Dunnett's multiple comparison test.

G,H Mitochondrial Ca^{2+} concentration was monitored by Rhod-2 AM at every 15 min. FCCP (200 μM) and antimycin A (50 μM) were used as pretreatments for 30 min. Data were normalized by data of DMSO before cold stress and the data at 60 min (H) are presented as mean ± SEM; $n = 3$, biological replicates, $^{****}P < 0.0001$, $^{**}P < 0.01$, by one-way ANOVA followed by Dunnett's multiple comparison test.

I-L Cytosolic Ca^{2+} concentration (I-J) and mitochondrial Ca^{2+} concentration (K-L) were monitored by Cal-520 AM and Rhod-2 AM, respectively, at every 15 min. BAPTA-AM (10 μM) were used as pretreatments for 30 min. Data were normalized by data of DMSO before cold stress, and the data at 60 min (J, L) are presented as mean ± SEM; $n = 4$, biological replicates, $^{*}P < 0.05$, by paired T-test.

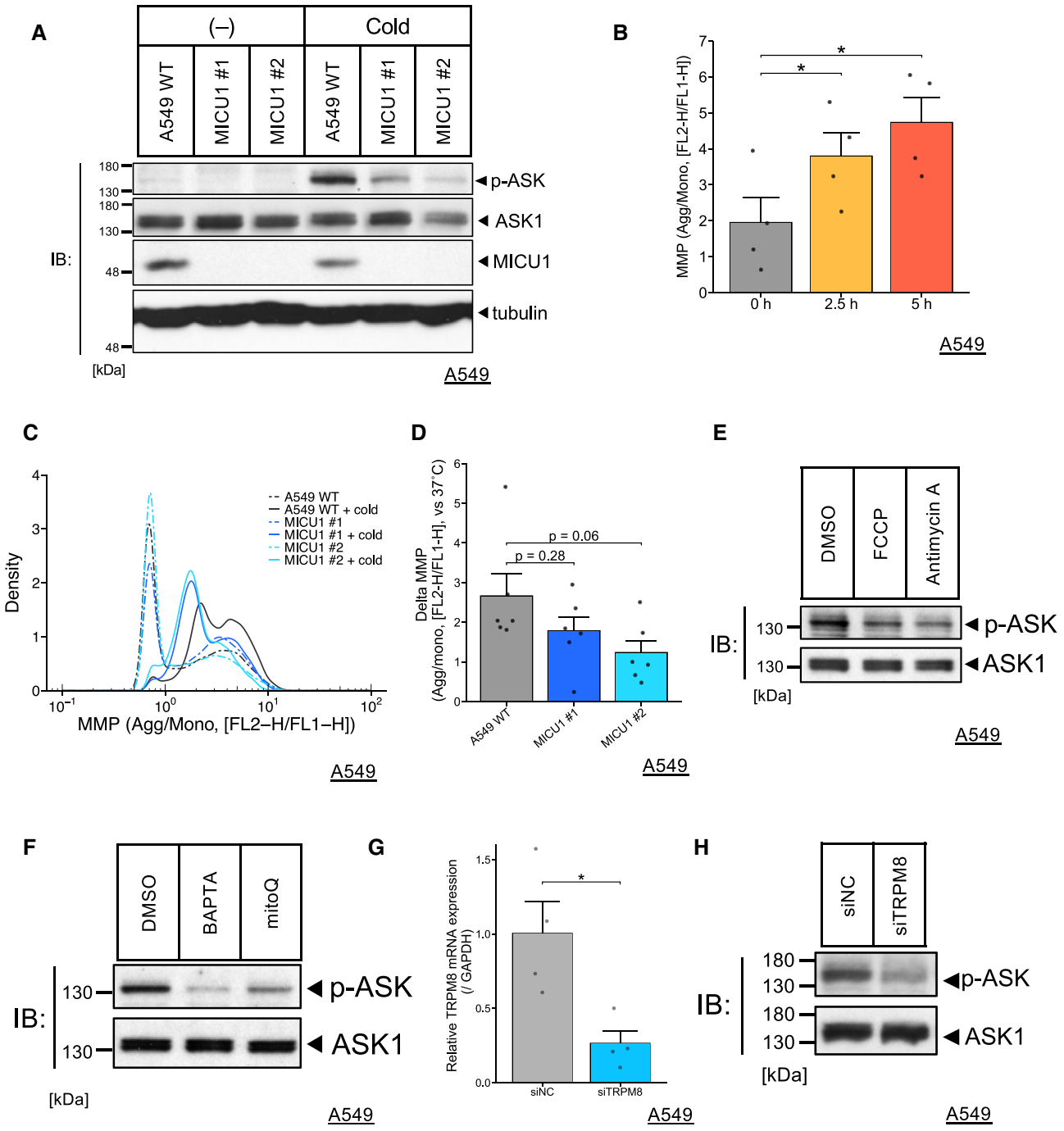

Figure EV4. ASK1 activity in various conditions and hyperpolarization of MMP under cold stress, related to Figs 1, 3 and 4.

A Immunoblots of endogenous phospho-ASK signals after cold stress for 5 h of the WT A549 cells, cloned MICU1-KO cells.

B Mitochondrial membrane potential (MMP) was measured by JC-1 after cold stress at the indicated time points. Quantification data for the WT A549 cells are presented as mean ± SEM; $n = 4$, biological replicates, $^*P < 0.05$, one-way ANOVA followed by William's test.

C,D Mitochondrial membrane potential was measured by JC-1 after cold stress for 5 h. Representative (C) and quantification data (D) are represented as mean ± SEM; $n = 6$, one-way ANOVA followed by Dunnett's test. Delta MMP was shown by the MMP of the WT A549 or MICU1 KO cells subtracted from that of each control condition.

E,F Immunoblots of endogenous phospho-ASK signals after cold stress for 5 h of the WT A549 cells. Inhibitors (FCCP: 200 μM, Antimycin A: 50 μM, BAPTA: 10 μM, mitoQ: 0.5 μM) were used as pretreatments for 30 min.

G TRPM8 mRNA was quantified by RT–qPCR. Data are presented as mean ± SEM; $n = 4$, $^*P < 0.05$, unpaired t-test.

H Immunoblots of endogenous phospho-ASK signals after cold stress for 5 h of the WT A549 cells or TRPM8-KD cells by siRNA transfection.

Figure EV5. MICU1 can be one of potential targets for cold-induced cell death, but MICU1 and cytosolic Ca^{2+} may not be involve in other ferroptotic cell death.

A LDH release was measured after DMSO or erastin (10 μM) treatment with DMSO or BAPTA-AM (10 μM) for 24 h. Data are presented as mean ± SEM; $n = 7$, biological replicates, ****$P < 0.0001$, n.s.: not significant, one-way ANOVA followed by Bonferroni's test.

B,C Cell viability was measured after erastin treatment as indicated concentrations for 24 hours in A549 cells and MICU1 #1/#2 KO A549 cells or Cas9-HT-1080 cells and Cas9-HT-1080 cells infected with lentivirus containing sgRNA of MICU1 #1/#2. Data are presented as mean ± SEM; $n = 3$ biological replicates.

D Immunoblots of MICU1 were present for knockout confirmation in Cas9-HT-1080 cells after lentivirus infection.

E Relative mRNA expression of MICU1 in normal human tissue was normalized by GAPDH mRNA expression. Data was obtained from Refex database.

F LDH release was measured after cold stress for 24 h in indicated cell lines. Data are presented as mean ± SEM; $n = 4$, biological replicates, **$P < 0.01$, *$P < 0.05$, one-way ANOVA followed by Dunnett's test.

G Immunoblots of MICU1 were present for knockdown confirmation in HepG2 cells after siRNA transfection.

H LDH release was measured after cold stress for 24 h in indicated cell lines. Data are presented as mean ± SEM; $n = 3$, biological replicates, ****$P < 0.0001$, one-way ANOVA followed by Dunnett's test.

I Immunoblots of MICU1 were present for knockdown confirmation in HEK293A cells after siRNA transfection.

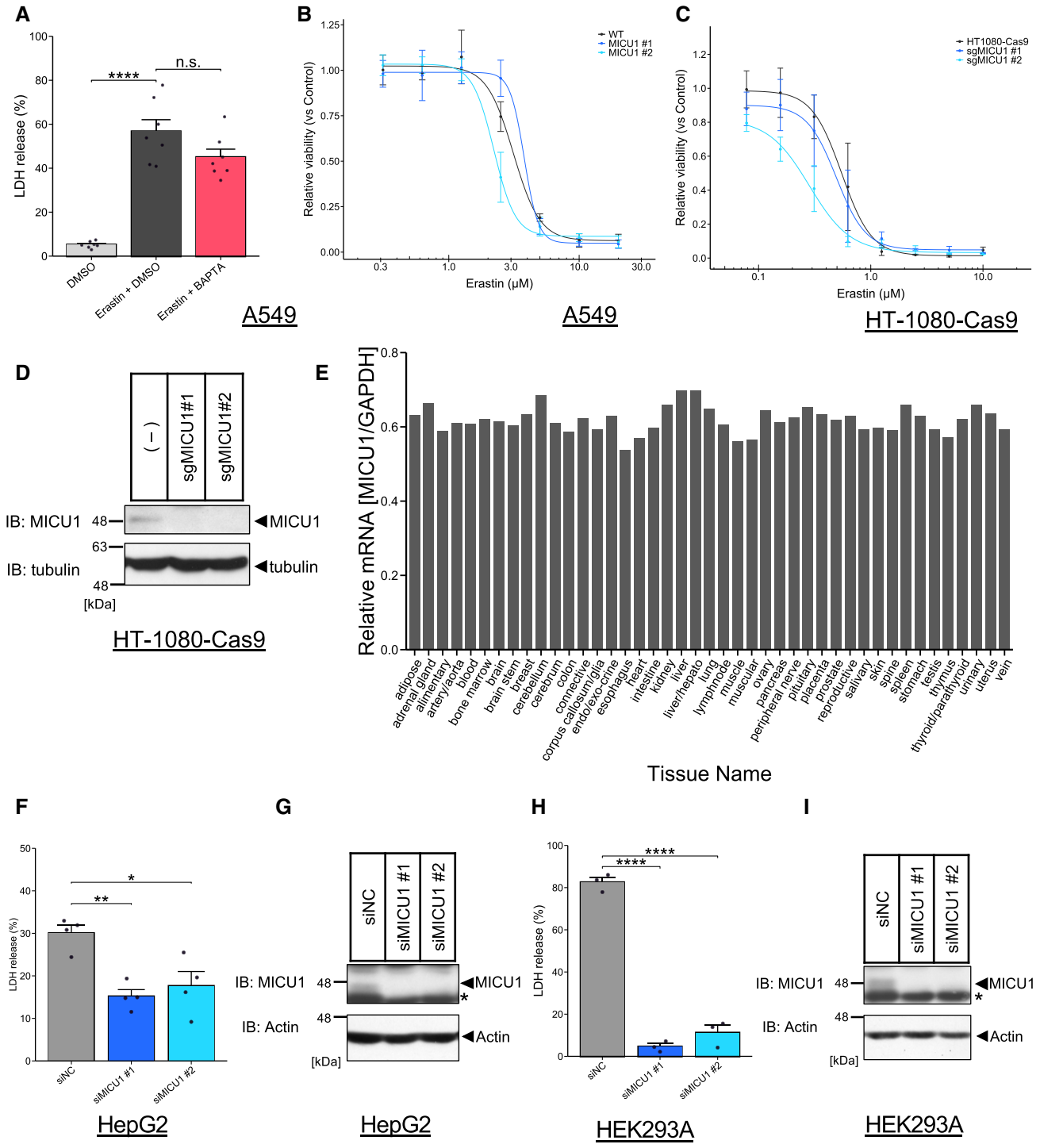

Figure EV5.