Expanded View Figures

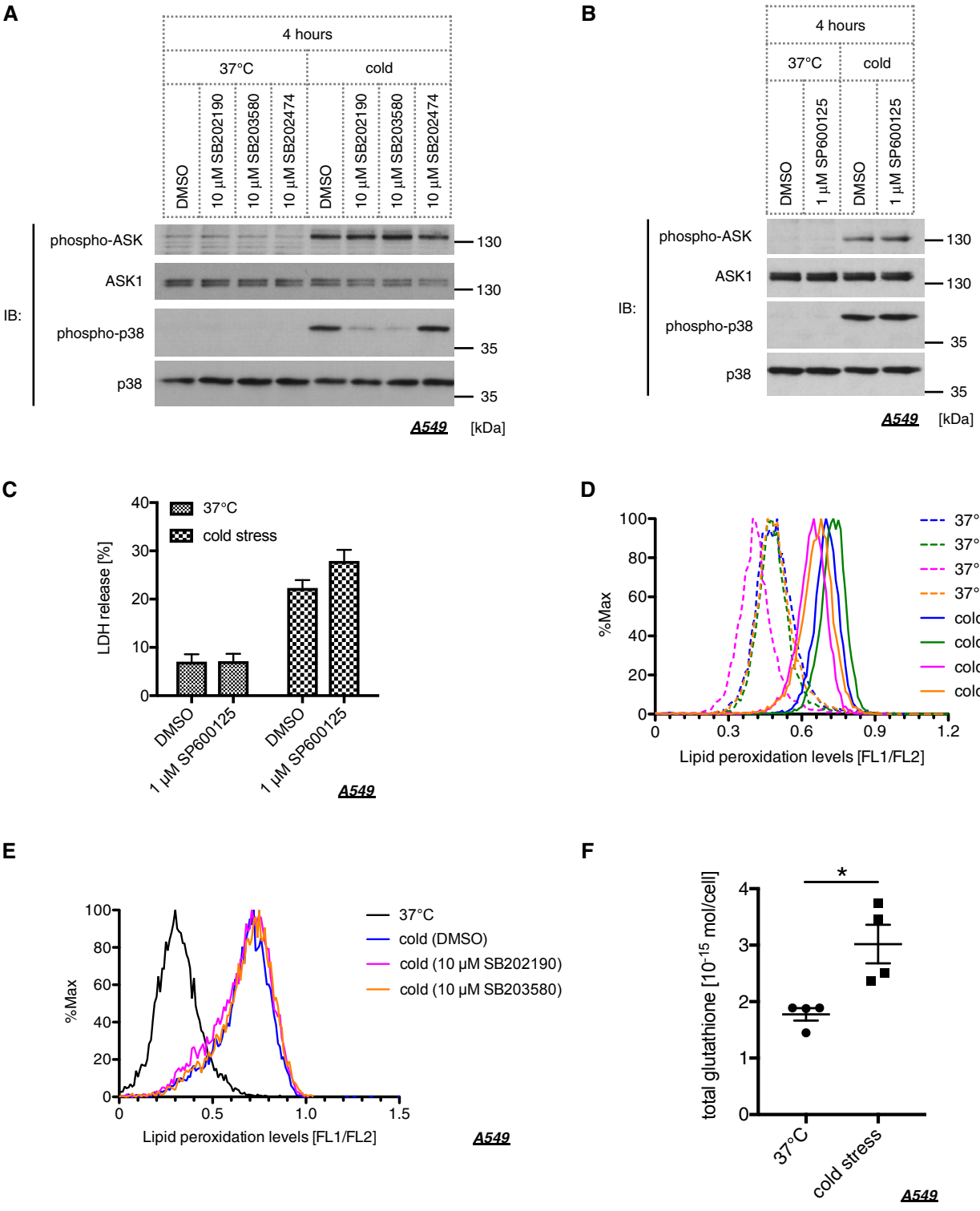

Figure EV1.

Figure EV1. Lipid peroxide-ASK1-p38 axis induces ferroptosis without mediating glutathione depletion.

A, B Cells were pretreated with p38 inhibitors (10 μM SB203580, 10 μM SB202190), their inactive analog (10 μM SB202474), and a JNK inhibitor (1 μM SP600125) 30 min before cold stress application. Endogenous phospho-ASK and phospho-p38 signals were assessed by Western blot analysis using A549 lysates treated with cold stress on ice for 4 h.

C Cells were pretreated with a JNK inhibitor (1 μM SP600125) 30 min before cold stress application. Cell death of A549 cells was determined by LDH assay after cold stress on ice for 8 h. Mean ± SEM, $N = 5$. Two-way ANOVA followed by Bonferroni's multiple comparisons test was performed.

D Lipid peroxidation levels in siRNA-treated A549 cells were determined by Bodipy 581/591 after 4 h of cold stress on ice.

E A549 cells were pretreated with p38 inhibitors (10 μM SB203580, 10 μM SB202190) for 30 min and then subjected to cold stress on ice for 4 h. Lipid peroxidation levels were determined by Bodipy 581/591.

F Cold stress on ice was applied to A549 cells for 4 h, and total glutathione was measured. Mean ± SEM, $N = 4$. *$P < 0.05$ by unpaired two-tailed Student's t-test with Welch correction.

Source data are available online for this figure.

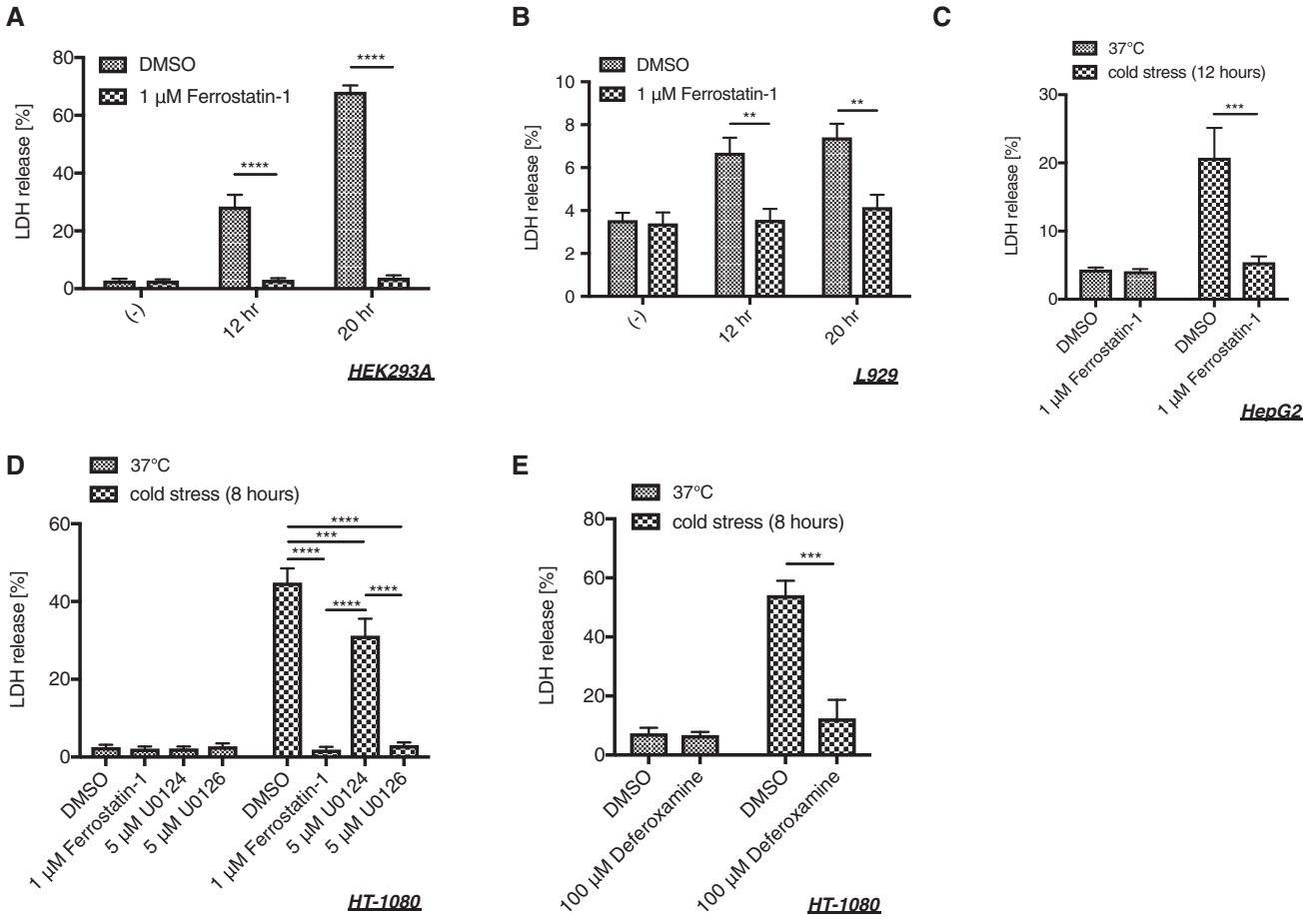

Figure EV2. Cold stress induces ferroptosis also in HEK293A, L929, HepG2, and HT-1080 cells.

A–E HEK293A (A), L929 (B), HepG2 (C), or HT-1080 (D, E) cells were pretreated with a lipid scavenger (1 μM ferrostatin-1), MEK inhibitor (5 μM U0126), its inactive analog (5 μM U0124), or an iron chelator (100 μM deferoxamine) for 30 min followed by cold stress on ice for the indicated time. Cell death was measured by LDH assay. Mean ± SEM, $N = 3$ (A, E), 5 (B, D), 6 (C). **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$ by two-way ANOVA followed by Bonferroni's (A–C, E) or Tukey's (D) multiple comparisons test. See also Appendix Table S2A.

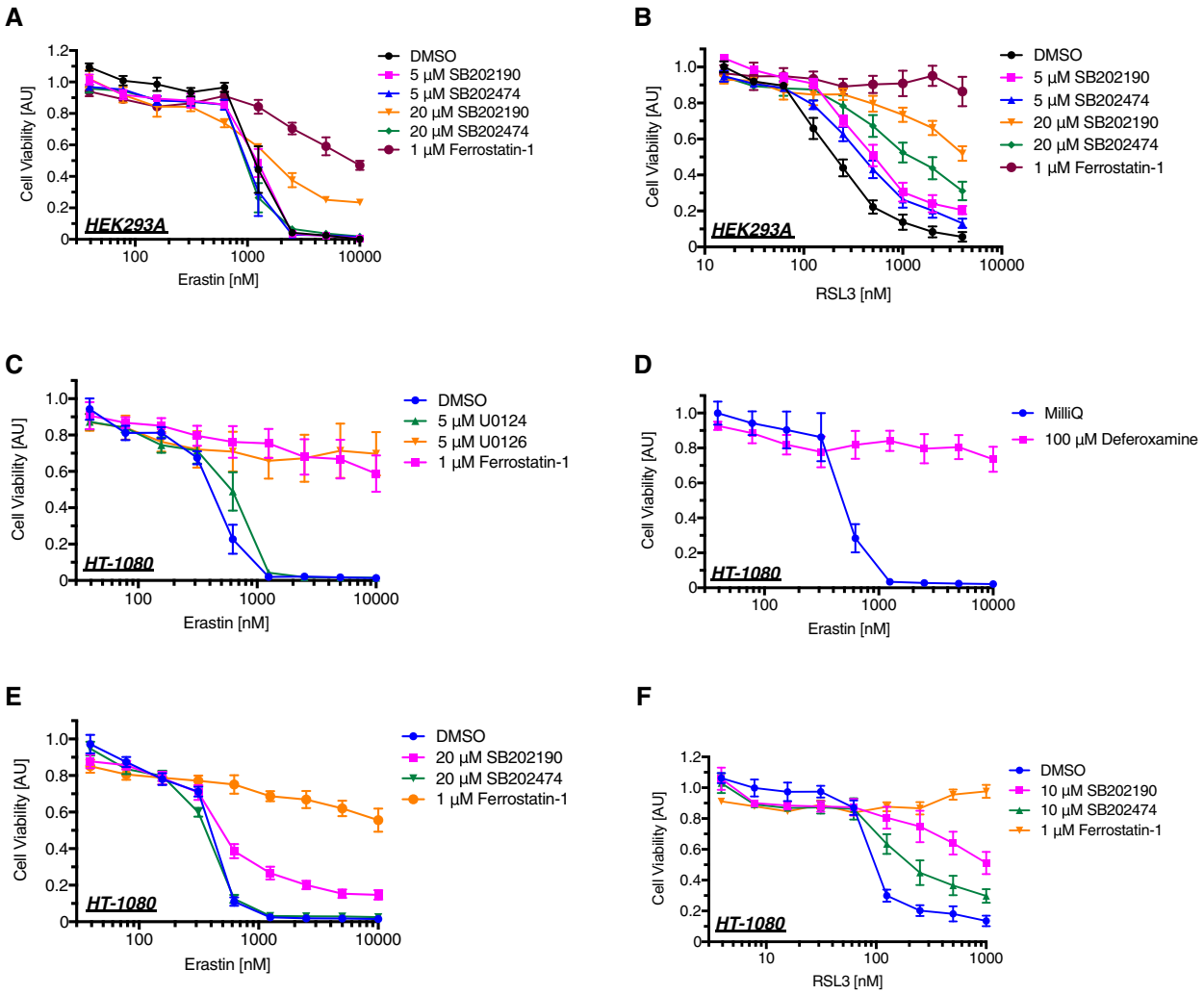

Figure EV3. p38 MAPK is partially involved in erastin- and RSL3-induced ferroptotic cell death.

A–F HEK293A (A, B) or HT-1080 (C–F) cells were treated with a lipid scavenger (1 μM ferrostatin-1), a MEK inhibitor (5 μM U0126), its inactive analog (5 μM U0124), an iron chelator (100 μM deferoxamine), p38 inhibitor (SB202190), or its inactive analog (SB202474), immediately followed by erastin or RSL3 treatment for 24 h at the indicated concentration. A Cell Counting Kit-8 was utilized to measure cell viability. Mean ± SEM, $N = 4$ (A–D), 5 (E), 3 (F). Two-way ANOVA followed by Tukey's multiple comparisons test. See also Appendix Table S2B–G.

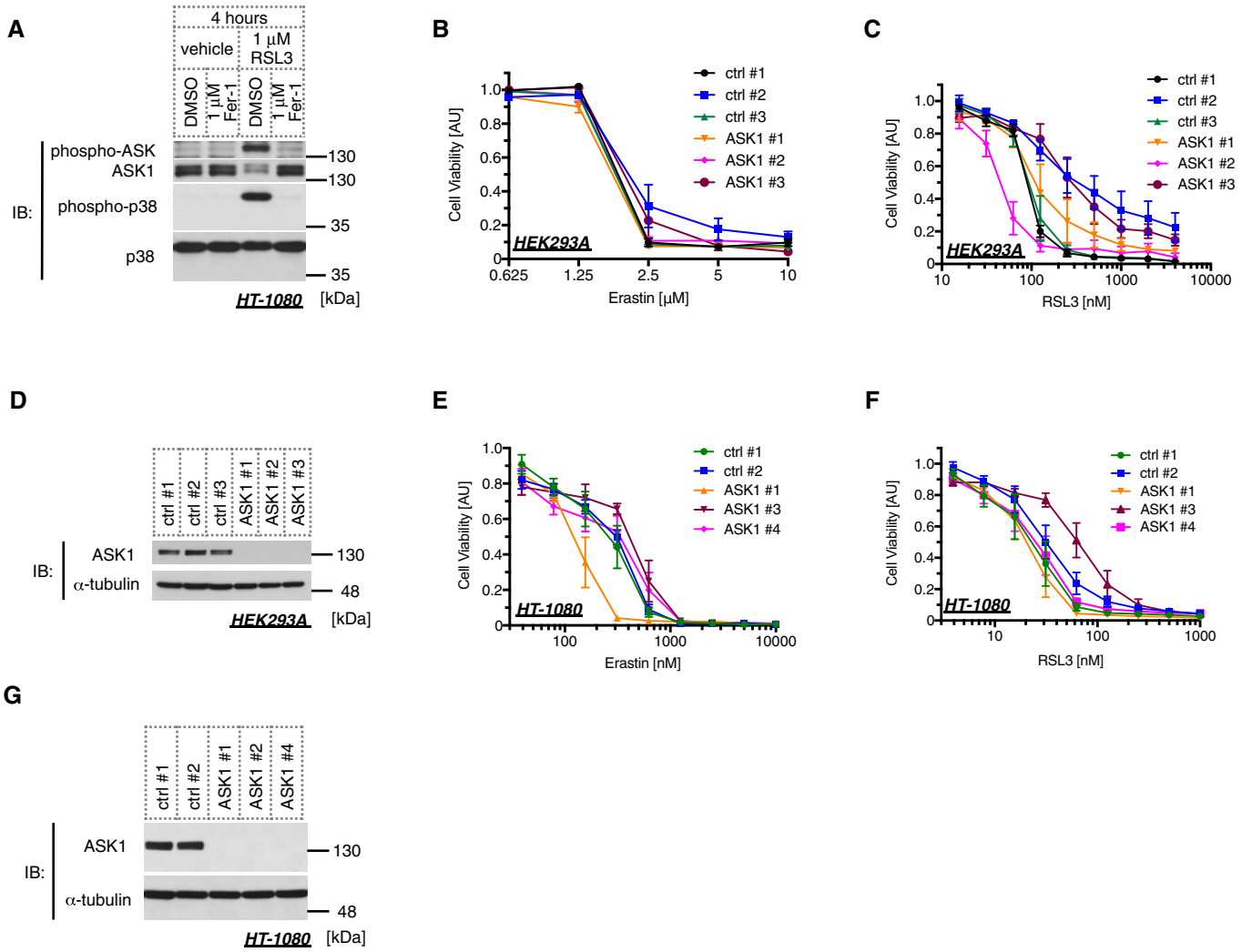

Figure EV4. ASK1 does not affect ferroptotic cell death in HEK293A and HT-1080 cells.

A Immunoblot of endogenous phospho-ASK and phospho-p38 signals in HT-1080 cells after 4 h of treatment with 1 μM RSL3 in the presence or absence of 1 μM ferrostatin-1. RSL3 administration was performed immediately after ferrostatin-1 treatment.

B, C, E, F HEK293A (B, C) or HT-1080 (E, F) cells were transfected with siRNAs followed by erastin (B, E) or RSL3 (C, F) treatment. Cells were reseeded a day prior to erastin or RSL3 treatment in order to match the number of cells among each sample. Cells were treated with erastin or RSL3 at the indicated concentration for 24 h. Cell viability was measured using the Cell Counting Kit-8. Mean ± SEM, $N = 5$ (B, F), 3 (C, E). Two-way ANOVA followed by Tukey's multiple comparisons test. See also Appendix Table S2H–K.

D, G Immunoblots of endogenous ASK1 and α-tubulin in HEK293A (D) or HT-1080 (G) cells for confirming the knockdown efficiency of the samples used in (B and C), and (E and F), respectively.

Source data are available online for this figure.

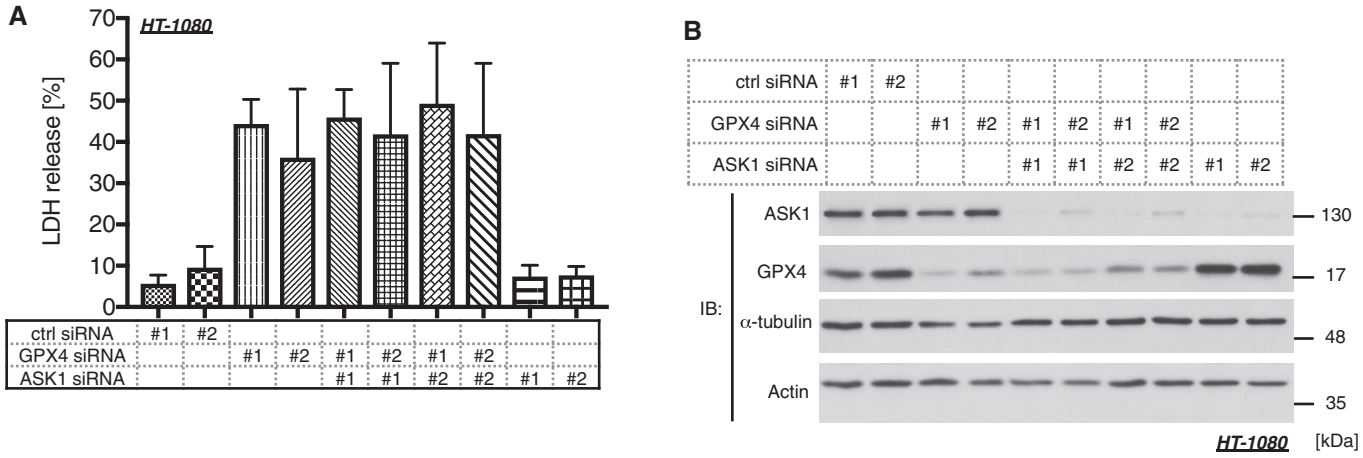

Figure EV5. ASK1 is not involved in GPX4 deficiency-dependent ferroptosis.

A Cell death of HT-1080 cells was examined by LDH assay 2 days after siRNA transfection. Mean ± SEM, $N = 4$. One-way ANOVA followed by Tukey's multiple comparisons test. See also Appendix Table S2L.

B Immunoblots of endogenous ASK1, GPX4, α-tubulin, and actin in HT-1080 cells for confirming the knockdown efficiency of the samples that was obtained 1 day after siRNA transfection.

Source data are available online for this figure.