# Tables

**Table S1. Significant genes from Cycle 3 of genome-wide BHK-21 screen (median log2 fold-change < -1 or > 1 and FDR < 0.1)**

| Gene | Median Log2FC | FDR |
|---|---|---|
| Rps29 | 1.3794 | 0.017327 |
| Dld | -1.7915 | 0.003094 |
| Eefsec | -2.1607 | 0.000707 |
| Gpx4 | -2.2215 | 0.000707 |
| Lias | -1.1881 | 0.011251 |
| Lipt1 | -1.1444 | 0.05562 |
| Pstk | -2.3621 | 0.000707 |
| Secisbp2 | -1.1041 | 0.000707 |
| Sepsecs | -2.3116 | 0.000707 |
| Ybey | -1.5789 | 0.017492 |

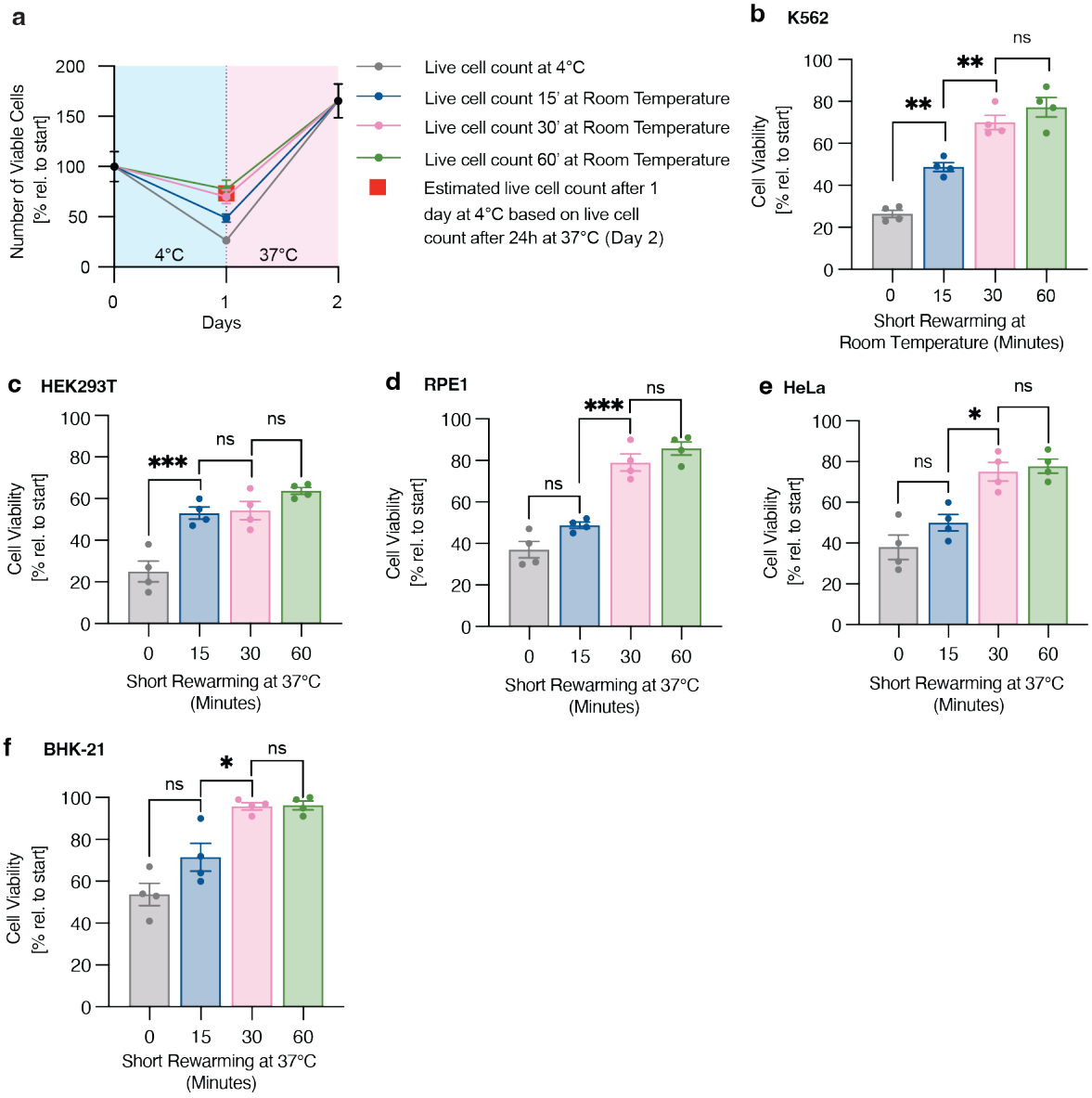
**Table S3. Significant genes from 15 days 4°C of genome-wide BHK-21 screen (median log2 fold-change < -0.5 or > 0.5 and FDR < 0.1)**

| Gene | Median Log2FC | FDR |
|---|---|---|
| LOC101827392 | 0.5431 | 0.066832 |
| Eefsec | -0.81913 | 0.00165 |
| Gpx4 | -1.5133 | 0.00165 |
| Secisbp2 | -0.74996 | 0.00165 |

**Table S4. Significant genes from Cycle 3, 4°C + ferrostatin-1 vs 4°C of genome-wide K562 screen (median log2 fold-change < -0.5 or > 0.5 and FDR < 0.1)**

| Gene | Median Log2FC | FDR |
|---|---|---|
| UBA3 | 0.59092 | 0.035754 |
| PSTK | 0.85375 | 0.000707 |
| GPX4 | 1.3025 | 0.000707 |
| CMIP | 0.83519 | 0.09946 |
| OXSM | 0.82371 | 0.003094 |
| HSCB | 1.0999 | 0.060891 |
| FTH1 | 1.4415 | 0.000707 |
| SEPHS2 | 0.98322 | 0.000707 |
| EEFSEC | 0.91866 | 0.000707 |
| FDXR | 1.2588 | 0.000707 |
| SEPSECS | 0.9745 | 0.000707 |

1226 **Supplement**

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1229 **Supplement 1. Permeability to trypan blue changes rapidly upon cell rewarming**

1230 **a**, Number of viable K562 cells based on trypan blue staining after one day at 4°C and

1231 subsequent rewarming for 24 hours at 37°C. Numbers are normalized to initial cell counts. Dots

1232 indicate viable cell number based on trypan blue staining of cells after incubation at room

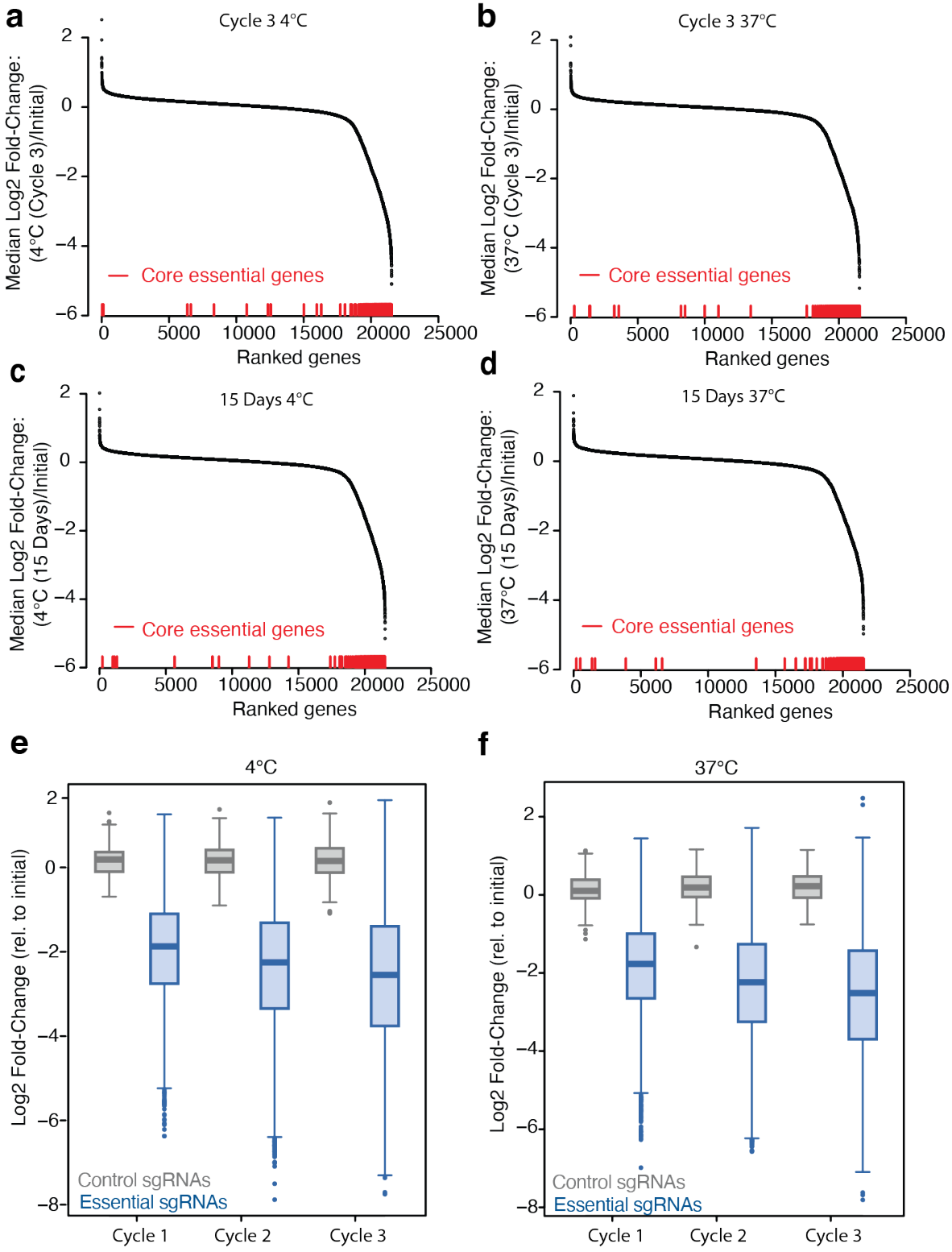
1233 temperature for 15, 30, or 60 minutes. Blue shaded regions indicate 4°C exposure and shaded

1234 pink regions indicate 37°C exposure. Red square indicates calculated cell counts after one day

1235 at 4°C based on the viable cell number measured after 24 hour rewarming. **b**, Viability of K562

1236 cells was assessed by trypan blue staining after incubation at room temperature for 0, 15, 30, or

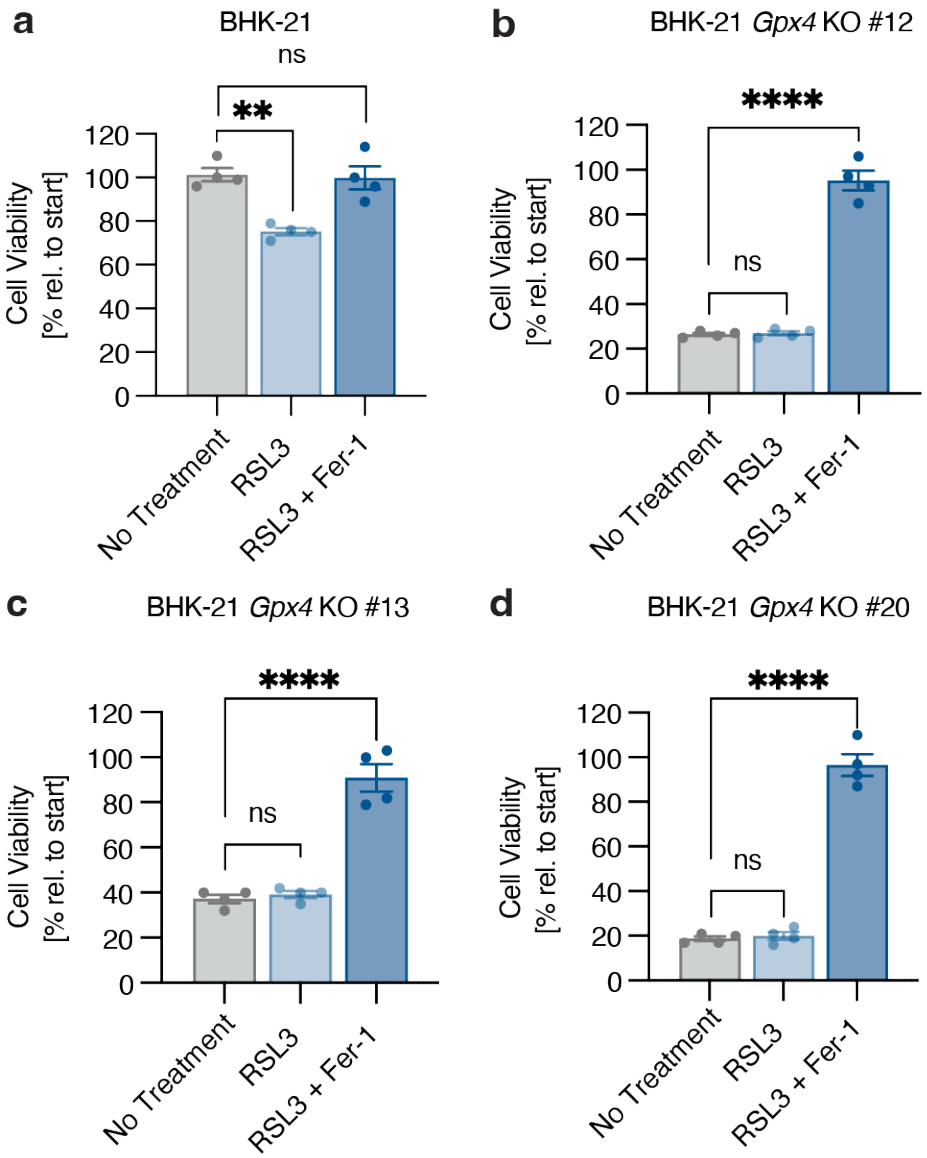
60 minutes following 24 hours at 4°C ($n$ = 4). Cells incubated at room temperature for 15 minutes show a significant increase in cell counts compared to cells counted immediately (**$P$ = 0.0016). Cells incubated at room temperature for 30 minutes show a significant increase in cell counts compared to a 15-minute incubation (**$P$ = 0.0023), while no significant difference in viability was observed between cells incubated for 30 or 60 minutes (ns, $P$ = 0.4059). **c-f**, Viability of cells was assessed by trypan blue staining after incubation at 37°C for 0, 15, 30, or 60 minutes following 24 hours at 4°C ($n$ = 4). **c**, HEK293T, **d**, RPE1, **e**, HeLa, **f**, BHK-21. No significant difference in cell viability was observed between cells incubated for 30 or 60 minutes for HEK293T (ns, $P$ = 0.3122), RPE1 (ns, $P$ = 0.5137), HeLa (ns, $P$ = 0.9735), and BHK-21 (ns, $P$ = 0.9998) cells. All values show mean ± SEM, with significance determined by one-way ANOVA adjusted for multiple comparisons by Tukey's HSD. *$P$ < 0.05; **$P$ < 0.01; ***$P$ < 0.001; ****$P$ < 0.0001; ns $P$ > 0.05.




**Supplement 2. Depletion of Core Essential Genes in Genome-Wide BHK-21 Screens**

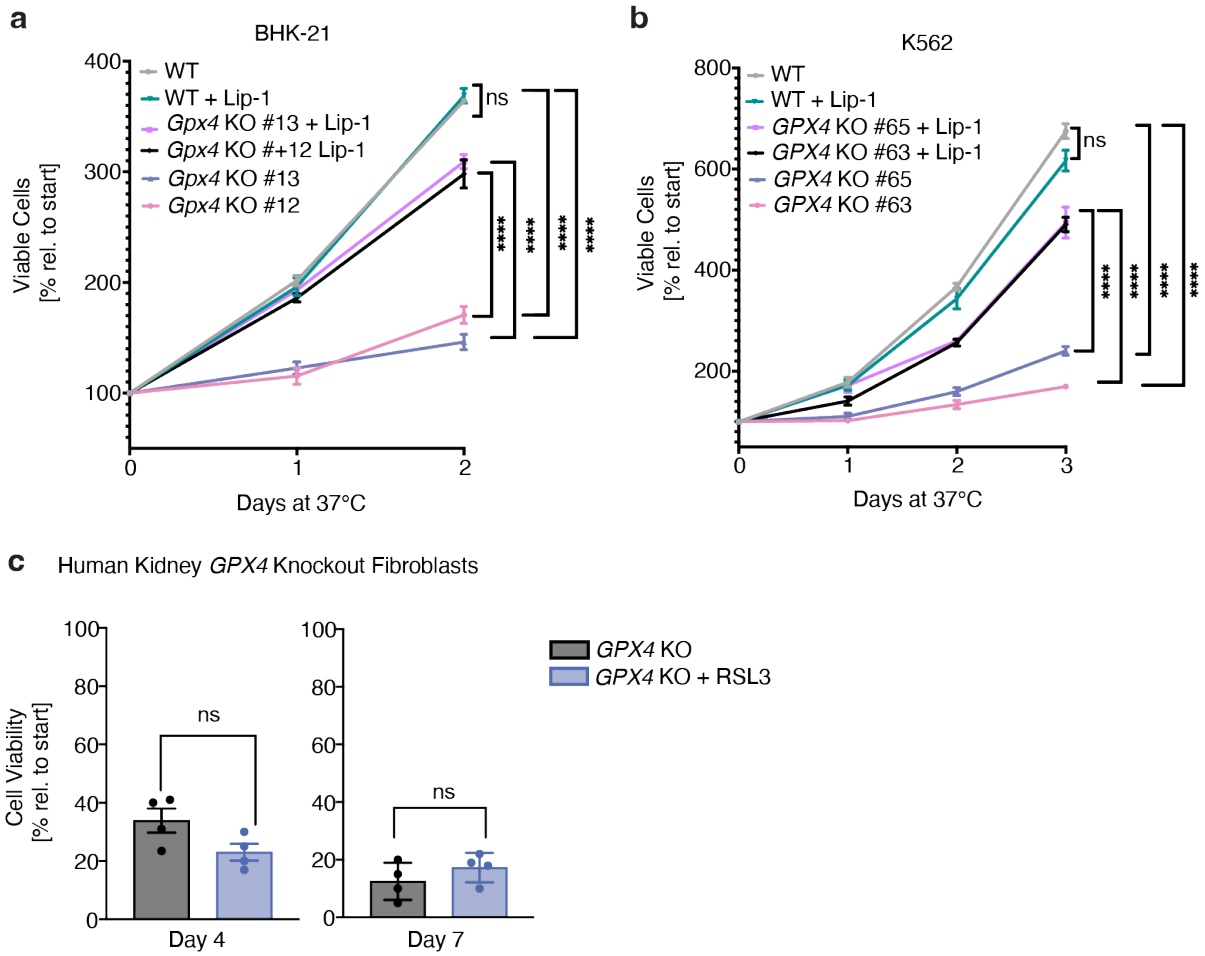
**a-d**, Genes ranked by median fold-change (log2) in genome-wide BHK-21 screens. **a**, after three cycles of cold exposure and rewarming (Cycle 3 4°C), **b**, matched constant 37°C control

condition (Cycle 3 37°C), **c**, after 15 days of 4°C exposure (15 Days 4°C), **d**, matched constant 37°C control condition (15 Days 37°C). Core essential genes[24] indicated in red are positioned below based on gene rank to demonstrate their depletion in each screen condition. **e-f**, Boxplots showing log2 fold change in representation for the population of control sgRNAs (gray; n = 250) or sgRNAs targeting core essential genes[24] (blue; n = 4635) over **e)** three cycles of cold exposure and rewarming (4°C) or **f)** constant 37°C control conditions. The line within each box represents the median, the bounds of each box represent the first and third quartiles, and the whiskers extend to the furthest data point within 1.5 times the interquartile range. A two-sided Kolmogorov-Smirnov test was used to test the difference between each pair of control/essential-gene-targeting sgRNA distributions (estimated p-value < 2.2e-16 for all six pairs in e and f).




**Supplement 3. RSL3 treatment has no effect on the viability of cold-exposed *Gpx4* KO BHK-21 cells.**

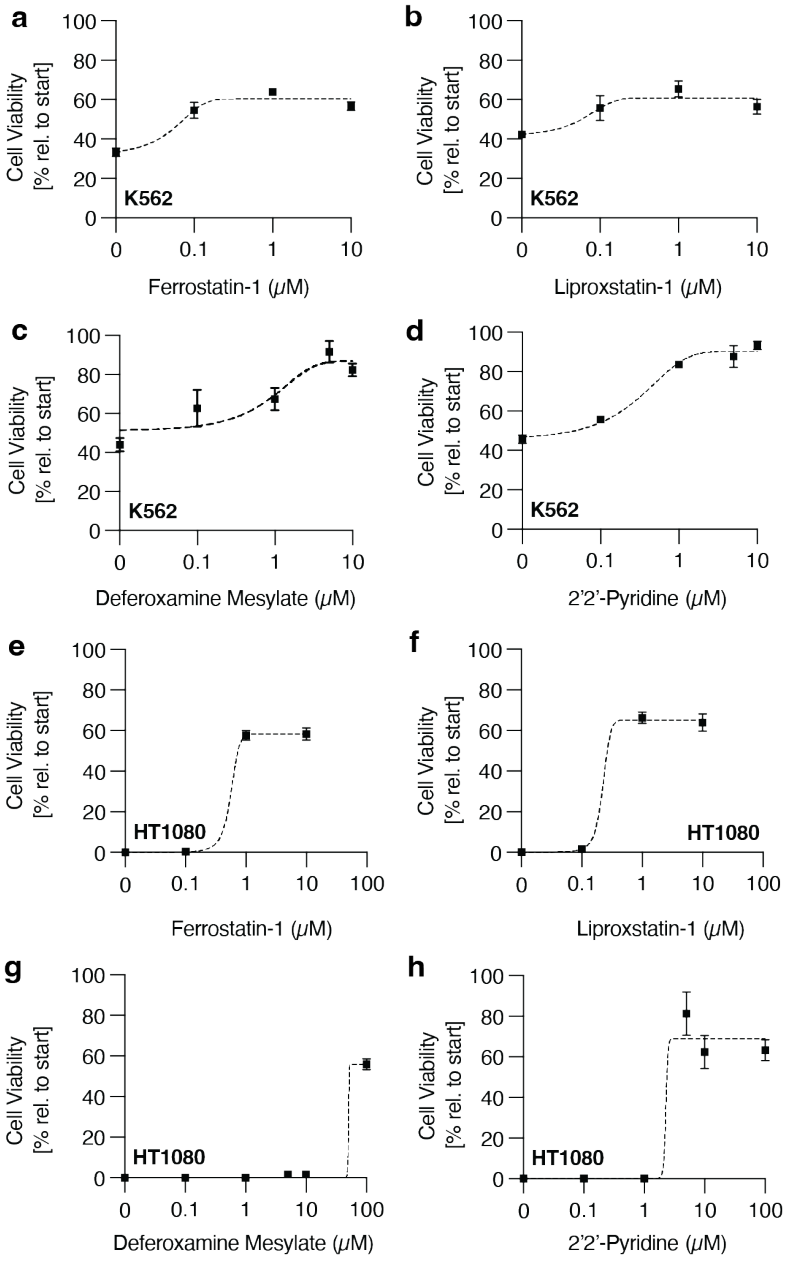
**a-d**, Wild-type BHK-21 cells **(a)** and three independent *Gpx4* KO BHK-21 clonal lines **(b-d)** were treated with RSL3 (1 μM) and ferrostatin-1 (Fer-1, 1 μM) as indicated for 24 hours at 4°C ($n$ = 4) prior to trypan blue staining. Wild-type BHK-21 cells show a significant decrease in cell viability when treated with RSL3 compared to no treatment (**$P$ = 0.0013), whereas *Gpx4* KO lines show no significant changes in viability (ns, $P$ > 0.05). All values show mean ± SEM, with significance measured by one-way ANOVA adjusted for multiple comparisons by Dunnett's test. *$P$ < 0.05; **$P$ < 0.01; ***$P$ < 0.001; ****$P$ < 0.0001; ns $P$ > 0.05.




**Supplement 4. Growth of BHK21 and K562 *GPX4* knockout cells and effects of RSL3 treatment on human kidney fibroblast *GPX4* knockout cells**

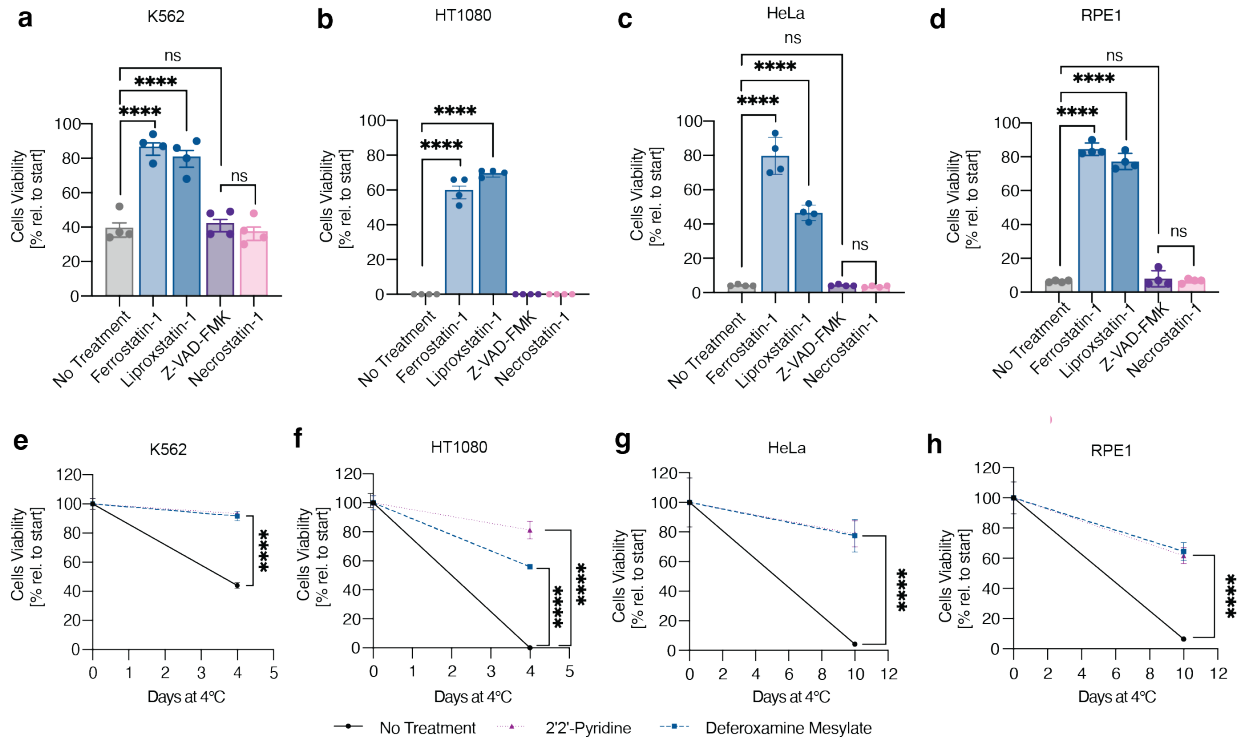
**a**, Viable K562 cells recorded as percentage relative to start based on trypan blue staining over the course of 3 days at 37°C. Cell growth is significantly decreased in *GPX4* K562 KO clones compared to WT K562 cells. Supplementation of liproxstatin-1 (2.5 μM) increases cell viability in *GPX4* KO cells and not WT cells at 37°C. **b**, Viable BHK-21 cells recorded as percentage relative to start based on trypan blue staining over the course of 2 days. Cell growth is significantly decreased in *Gpx4* BHK-21 KO cells compared to WT BHK-21 cells. Supplementation of liproxstatin-1 (2.5 μM) increases cell viability in *Gpx4* KO cell and not WT cells at 37°C. **c**, Human kidney GPX4 KO cells were placed at 4°C and left untreated or treated with RSL3 (1 μM) for 4, and 7 days (n = 4). Treatment with RSL3 does not confer significant additional death (n=4 per timepoint and condition) as measure by two-tailed t-test. All values show mean ± SEM, with significance measured by one-way ANOVA adjusted for multiple

comparisons with Tukey's HSD unless otherwise specified. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$; ns $P > 0.05$.




**Supplement 5. Ferroptosis inhibitors and iron chelators increase cold cell viability in a dose-dependent manner**

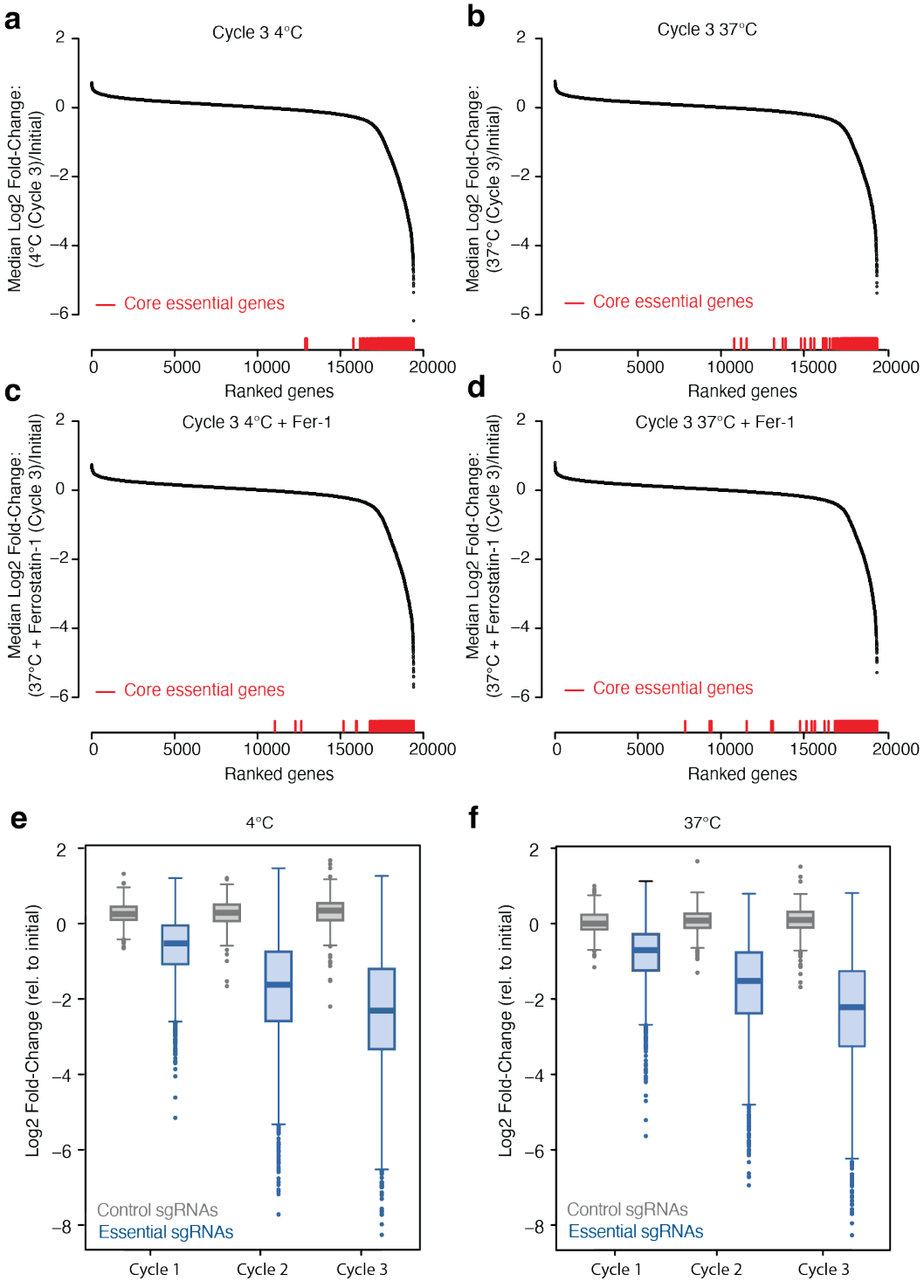
**a-h**, K562 **(a-d)** and HT1080 **(e-h)** cells were treated with varying concentrations of the ferroptosis inhibitors, ferrostatin-1 and liproxstatin-1, and iron chelators, deferoxamine and 2'2'-pyridine, for four days at 4°C prior to assaying cell viability by trypan blue staining ($n$ = 3). All values show mean ± SEM.




**Supplement 6. Cells derived from non-hibernators undergo cold-induced ferroptotic cell death**

**a**, K562 cells were treated with ferrostatin-1 (1 μM) (****$P$ < 0.0001), liproxstatin-1 (1 μM) (****$P$ < 0.0001), Z-VAD-FMK (1 μM) (ns, $P$ = 0.9760), or necrostatin-1 (1 μM) (ns, $P$ = 0.9835) for 4 days at 4°C prior to trypan blue staining ($n$ = 4). **b**, HT1080 cells treated with ferrostatin-1 (1 μM) (****$P$ < 0.0001), liproxstatin-1 (1 μM) (****$P$ < 0.0001), Z-VAD-FMK (1 μM), or necrostatin-1 (1 μM) for 4 days at 4°C prior to trypan blue staining ($n$ = 4). **c**, HeLa cells treated with ferrostatin-1 (1 μM) (****$P$ < 0.0001), liproxstatin-1 (1 μM) (****$P$ < 0.0001), Z-VAD-FMK (1 μM) (ns, $P$ > 0.9999), or necrostatin-1 (1 μM) (ns, $P$ = 0.9987) for 10 days at 4°C prior to trypan blue staining ($n$ = 4). **d**, RPE1 cells treated with ferrostatin-1 (1 μM) (****$P$ < 0.0001), liproxstatin-1 (1 μM) (****$P$ < 0.0001), Z-VAD-FMK (1 μM) (ns, $P$ = 0.9291), or necrostatin-1 (1 μM) (ns, $P$ > 0.9999) for 10 days at 4°C prior to trypan blue staining ($n$ = 4). **e**, K562 cells treated with deferoxamine mesylate (5 μM) (***$P$ = 0.0002) or 2'2'-pyridine (10 μM) (****$P$ < 0.0001) for 4 days at 4°C prior to trypan blue staining ($n$ = 3). **f**, HT1080 cells treated with deferoxamine mesylate (100 μM) (****$P$ < 0.0001) or 2'2'-pyridine (5 μM) (***$P$ = 0.0002) for 4 days at 4°C prior to trypan blue staining ($n$ = 3). **g**, HeLa cells treated with deferoxamine mesylate (5 μM) (****$P$ < 0.0001) or 2'2'-pyridine (5 μM) (****$P$ < 0.0001) for 10 days at 4°C prior to trypan blue staining ($n$ = 3). **h**,

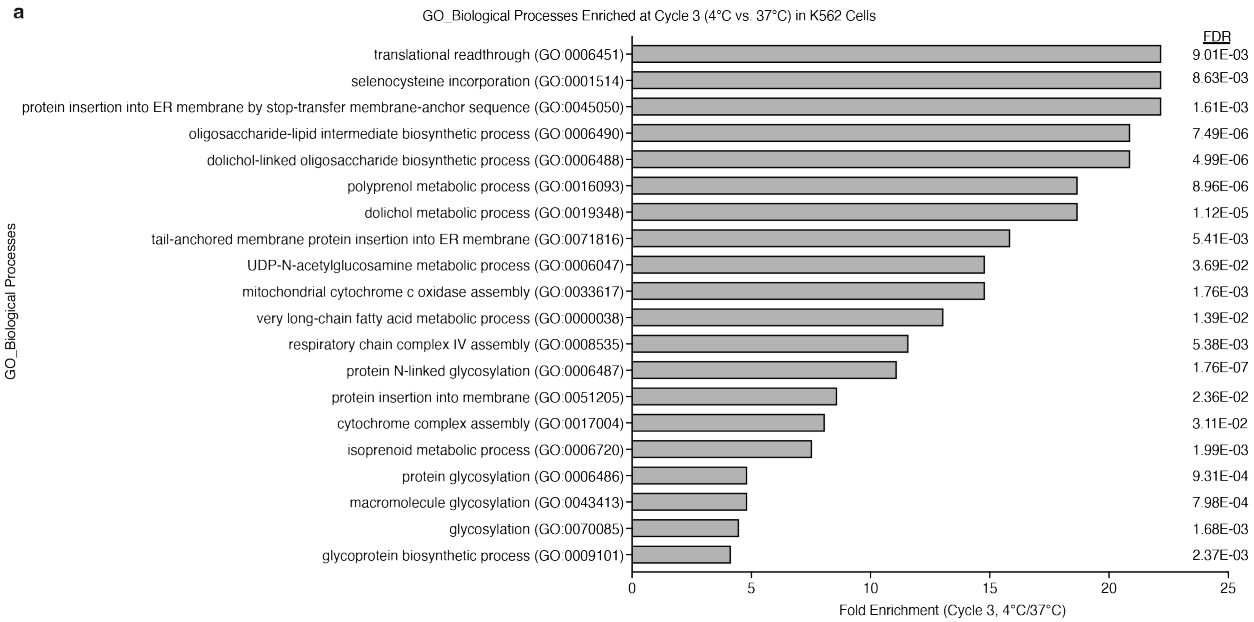
RPE1 cells treated with deferoxamine mesylate (100 μM) (****$P < 0.0001$) or 2'2'-pyridine (5 μM) (***$P < 0.0001$) for 10 days at 4°C prior to trypan blue staining ($n = 3$). All values show mean ± SEM, with significance measured one-way ANOVA adjusted for multiple comparisons by Dunnett's test. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$; ns $P > 0.05$.




**Supplement 7. Depletion of Core Essential Genes in Genome-Wide K562 Screens**

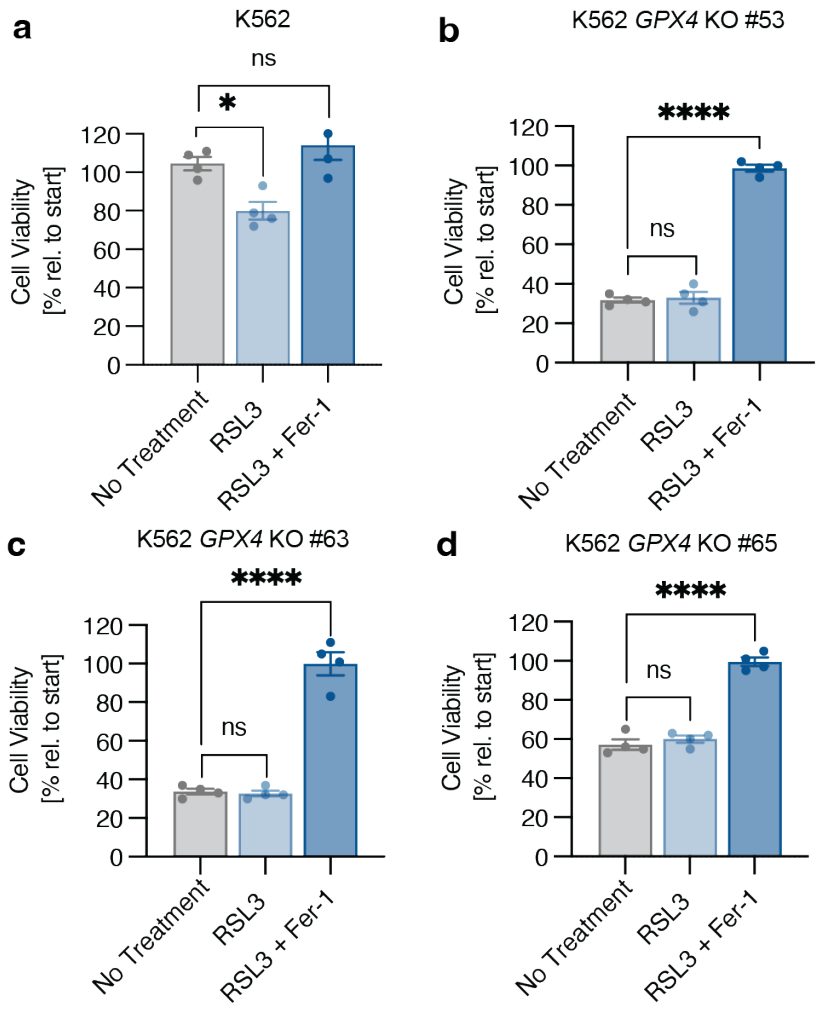
**a-d**, Genes ranked by median fold-change (log2) in genome-wide K562 screens. **a**, after three cycles of cold exposure (Cycle 3 4°C), **b**, matched constant 37°C control condition (Cycle 3

37°C), **c**, three cycles of cold exposure with 1 μM ferrostatin-1 (Cycle 3 4°C + Fer-1), **d**, matched constant 37°C control condition with 1 μM ferrostatin-1 (Cycle 3 37°C + Fer-1). Core essential genes[24] (red bars) are positioned below based on gene rank to demonstrate their depletion in each screen condition. **e-f**, Boxplots showing the log2 fold change in representation for the population of control sgRNAs (gray; n = 500) or sgRNAs targeting core essential genes[24] (blue; n = 3219) over **e)** three cycles of cold exposure (4°C) or **f)** constant 37°C control conditions. The line within each box represents the median, the bounds of each box represent the first and third quartiles, and the whiskers extend to the furthest data point within 1.5 times the interquartile range. A two-sided Kolmogorov-Smirnov test was used to test the difference between each pair of control/essential-gene-targeting sgRNA distributions (estimated p-value < 2.2e-16 for all six pairs in e and f).



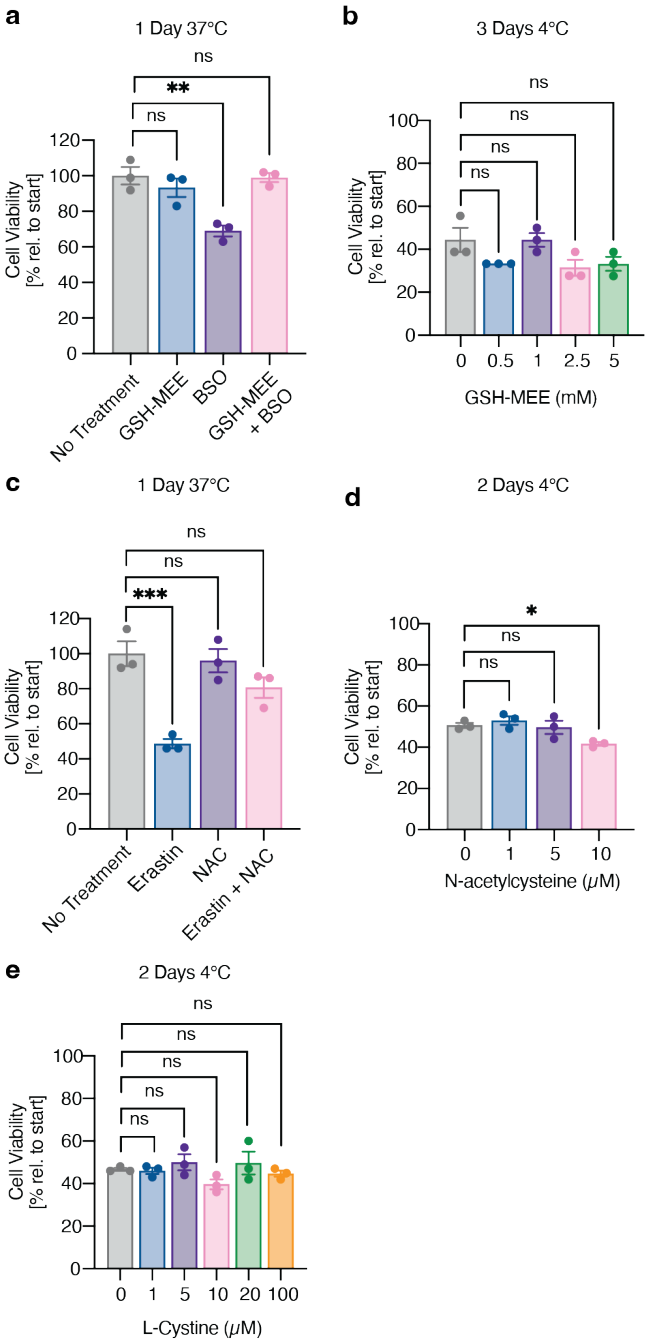

**Supplement 8. Top 20 enriched pathways at Cycle 3 (4°C vs. 37°C) in K562 cells**

**a**, Graphical representation of the top 20 enriched, differentially expressed gene sets (204 genes; FDR < 0.1) from the genome-scale CRISPR-Cas9 screen in K562 cells (Cycle 3 4°C vs. 3 passages at 37°C). GO_Biological Processes Panther overrepresentation test was used to determine enriched gene sets. Functional annotation analysis of the selectively required genes identified pathways related to translational readthrough, selenocysteine incorporation, protein insertion into the ER, glycosylation, fatty acid metabolism, and mitochondrial respiration. A full list of pathways is provided in Table S6.




**Supplement 9. RSL3 treatment has no effect on the viability of cold-exposed *GPX4* KO K562 cells.**

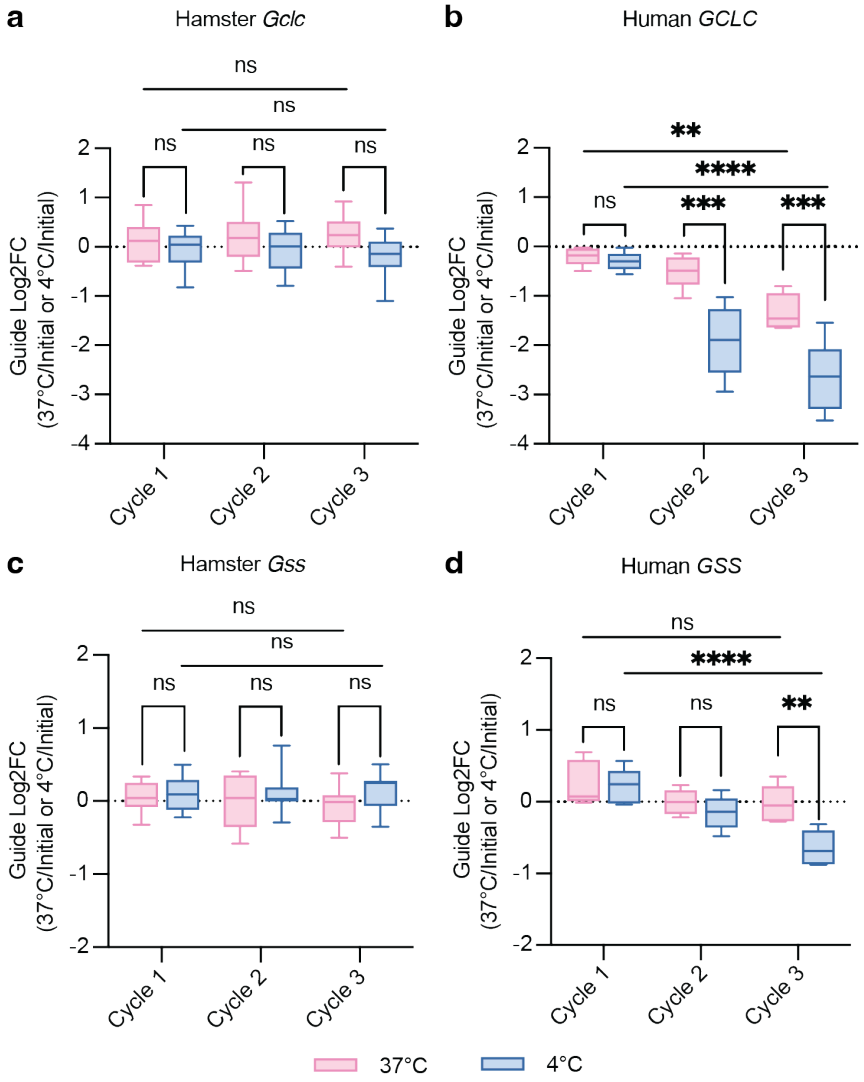
**a-d**, Wild-type K562 cells **(a)** and three independent *GPX4* KO K562 clonal lines **(b-d)** were treated with RSL3 (1 μM) and ferrostatin-1 (Fer-1, 1 μM) as indicated for 8 hours at 4°C ($n$ = 4) prior to trypan blue staining. Wild-type K562 cells show a significant decrease in cell viability when treated with RSL3 compared to no treatment (*$P$ = 0.0213), whereas *GPX4* KO lines show no significant changes in viability (ns, $P$ > 0.05). All values show mean ± SEM, with significance measured by one-way ANOVA adjusted for multiple comparisons by Dunnett's test. *$P$ < 0.05; **$P$ < 0.01; ***$P$ < 0.001; ****$P$ < 0.0001; ns $P$ > 0.05.




**Supplement 10. Cell permeable glutathione and glutathione precursors do not increase cold cell viability**

**a,** K562 cells were placed at 37°C and treated with cell permeable glutathione GSH-MEE (1 μM) and/or the glutathione synthesis inhibitor buthionine sulfoximine (BSO, 1 μM) for 1 day ($n$ = 3). Treatment with BSO resulted in increased cell death (**$P$ = 0.0018) that was rescued by GSH-MEE (ns, $P$ = 0.9961). **b,** Treatment with GSH-MEE has no effect on K562 cold-induced death

after 3 days at 4°C as measured by trypan blue staining ($n$ = 3, 5 μM; ns, $P$ = 0.1558). **c,** K562 cells were placed at 37°C and treated with ferroptosis inducer Erastin (10 μM) and N-acetylcysteine (NAC, 10 μM) for 1 day ($n$ = 3). Treatment with Erastin resulted in increased cell death (***$P$ = 0.0006) that was rescued by NAC (ns, $P$ = 0.1091). **d,** Treatment with N-acetylcysteine does not increase cold cell viability in K562 cells after 2 days at 4°C as measured by trypan blue staining ($n$ = 3, 10 μM; *$P$ = 0.0352). **e,** Treatment with L-Cystine does not increase cold cell viability in K562 cells after 2 days at 4°C as measured by trypan blue staining ($n$ = 3, 100 μM; ns, $P$ = 0.9843). All values show mean ± SEM, with significance measured by one-way ANOVA adjusted for multiple comparisons by Dunnett's test. *$P$ < 0.05; **$P$ < 0.01; ***$P$ < 0.001; ****$P$ < 0.0001; ns $P$ > 0.05.




**Supplement 11. Glutathione biosynthesis genes show increased depletion in cold-exposed K562 cells**

**a,c**, Log2 fold-change (log2FC) of 10 guides per targeted gene in BHK-21 genome-wide screen, showing guide depletion over three cycles of cold exposure and rewarming. **a**, *Gclc*, **c**, *Gss*. **b**, **d**, Log2 fold-change (log2FC) of 5 guides per targeted gene in K562 genome-wide screen, showing significant guide depletion over three cycles of cold exposure. **b**, *GCLC*, **d**, *GSS*. Significance between Cycle 1 versus Cycle 3 is measured by two-way ANOVA adjusted for multiple comparisons by Dunnett's test. Significance between 37°C and 4°C for each cycle is measured by two-way ANOVA adjusted for multiple comparisons by Bonferroni's test. **P* < 0.05; ***P* < 0.01; ****P* < 0.001; *****P* < 0.0001; ns *P* > 0.05.