

Supplemental figure legends

Fig. S1. Metabolome analysis revealed glutathione synthesis pathway is retained in hamster cells under cold and nutrient-deprived condition

a Amount of metabolites related to glutathione synthesis before or under cold temperature (4°C) and nutrient deprivation. **b** Amount of nucleotides before or under cold temperature (4°C) and nutrient deprivation. **c** Amount of amino acids before or under cold temperature (4°C) and nutrient deprivation. **d** Amount of NAD, NADH, NADPH before or under cold temperature (4°C) and nutrient deprivation. **e** Hierarchical clustering of metabolome profiles. Representative metabolites are shown. One-way ANOVA with the Dunnett's multiple comparison test compared to day0 in C-F, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$

Fig. S2 Expression levels of Gpx4 in HT1080, HapT1 and liver tissues of mice and hamsters

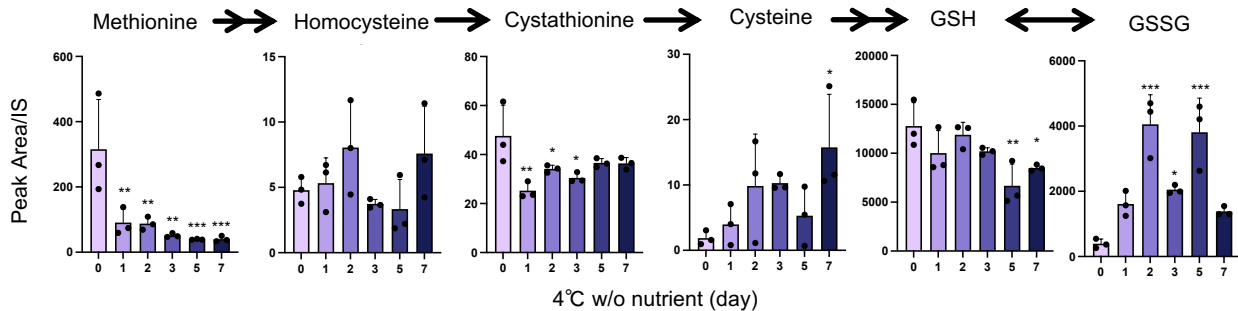
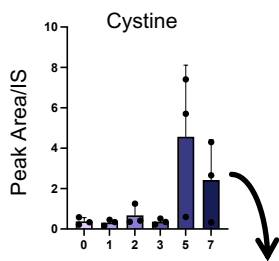
a Immunoblot of Gpx4 and β -Actin proteins in HT1080 and HapT1. **b** Immunoblot of Gpx4 and β -Actin proteins in mouse and hamster livers (N = 4). **c** Immunoblot of Gpx4 and β -Actin proteins in HT1080 and HapT1 under cold temperature (4°C) for indicated period. Signals were quantified and represented as bar plot in the right panel. **d** Immunoblot of Gpx4 and β -Actin proteins in livers of hamsters in the states of non-hibernation (room temperature, RT), periodic arousal (PA) and deep torpor (DT) (N = 3 or 4). Signals were quantified and represented as box plot in the right panel.

Fig. S3 Biotpterin and CoQ reduction pathways are required for short-term cold-resistance under Gpx4 dysfunction in hamster cells, related to Fig. 5

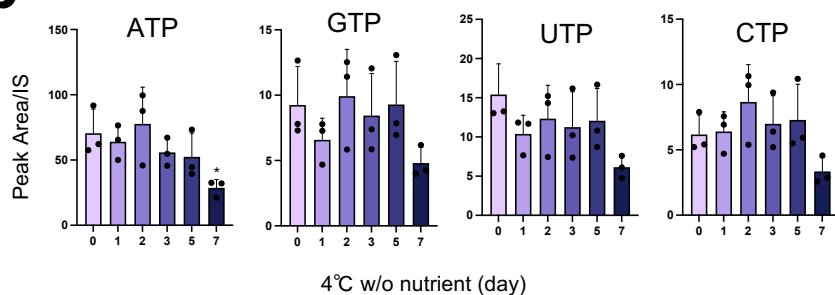
a - c The proportion of dead cells during cold culture (4°C) culture in the each HapT1 cell population infected with lentivirus expressing SpCas9 and non-targeting sgRNA (sgNT) or sgRNA for FSP1 (**a**) or Dhodh (**b**) or Gch1 (**c**) in the presence or absence of 6 μ M ML210 (One-way ANOVA with the Tukey's multiple comparison test, $p < 0.05$). **d** Assessment of knock-out efficiency of Spr in HapT1 cells. Immunoblots of Spr and β -Actin proteins in HapT1 cell populations infected with lentivirus vectors expressing SpCas9 and sgNT or sgRNA targeting Spr. **e** Assessment of knock-out efficiency of Pts in HapT1 cells. Quantitative RT-PCR of Spr and β -Actin mRNA in HapT1 cell populations infected with lentivirus vectors expressing SpCas9 and sgNT or sgRNA targeting Pts. Note that Pts mRNA was decreased in the cells introduced with sgPts presumably through nonsense mediated RNA decay pathway. **f** The proportion of dead

cells after two-day cold culture in HapT1 infected with lentivirus expressing SpCas9 and sgNT or sgRNA for Spr or Pts and treated with 2 μ M RSL3 or 6 μ M ML210 determined by LDH assay (One-way ANOVA with the Dunnett's multiple comparison test, **p < 0.01, ***p < 0.001). **g** The proportion of dead cells during cold culture in HapT1 infected with lentivirus expressing SpCas9 and sgNT or sgRNA for Gch1 with or without 100 μ M BH2 and 4 μ M methotrexate (MTX) determined by LDH assay (N = 3 wells).

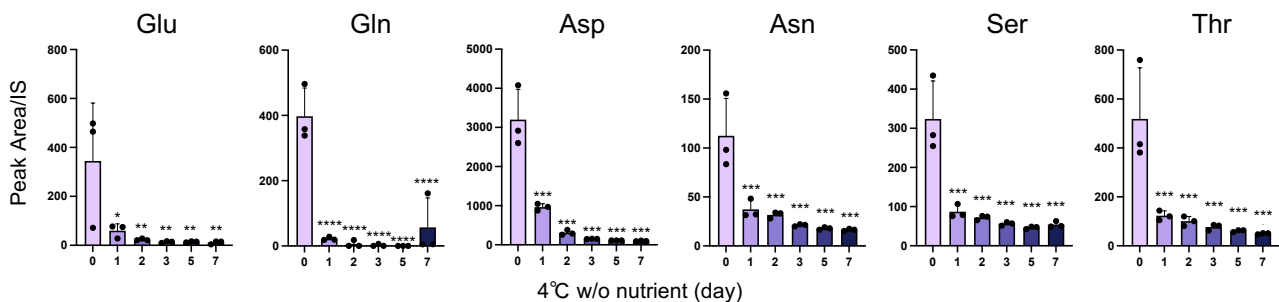
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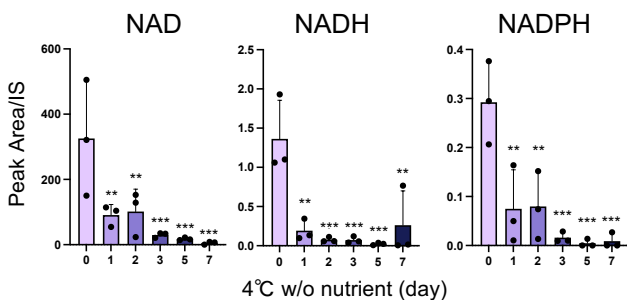
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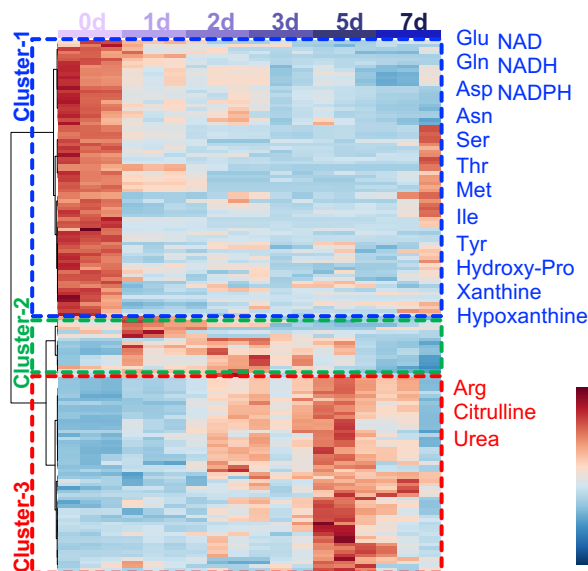
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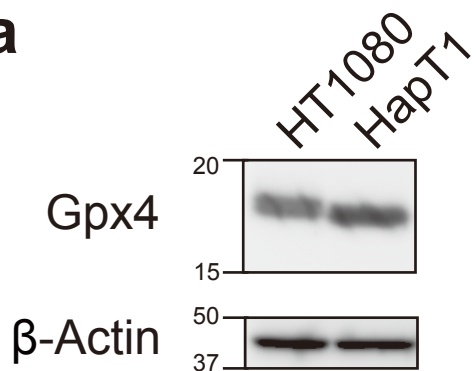
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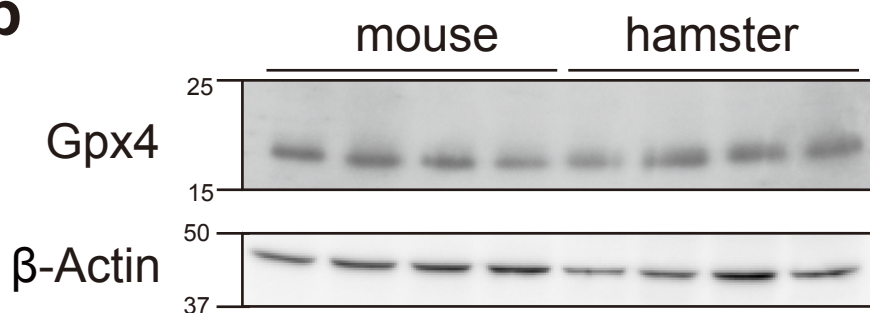
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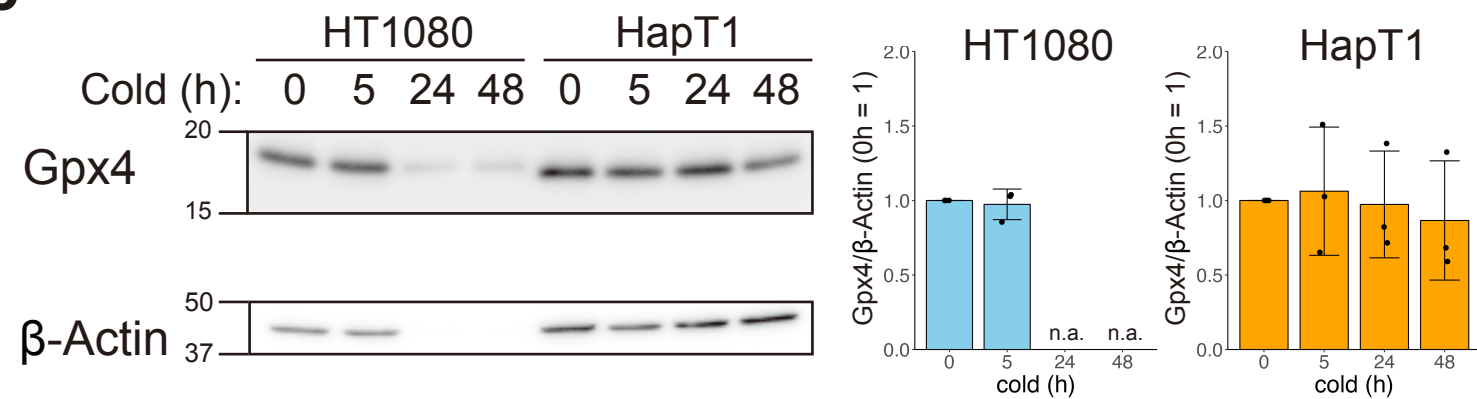
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b



c



d

