Supplementary data to:

Altered protein S-glutathionylation identifies a potential mechanism of resistance to acetaminophen-induced hepatotoxicity.

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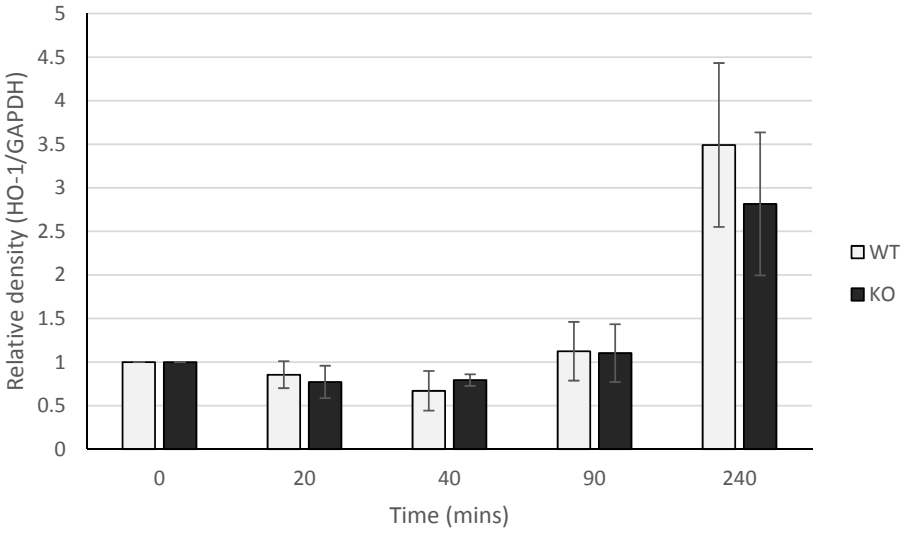
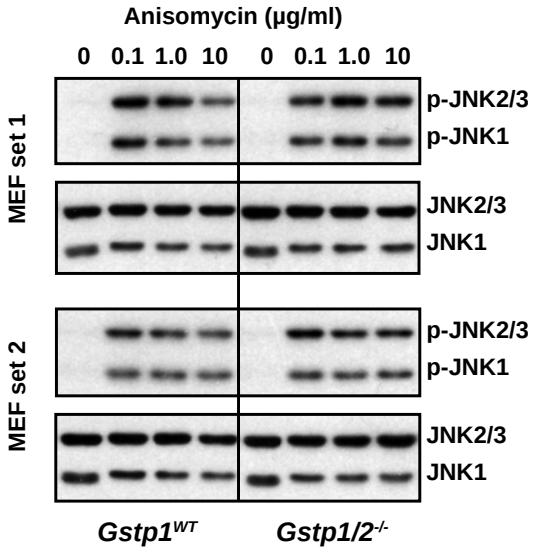


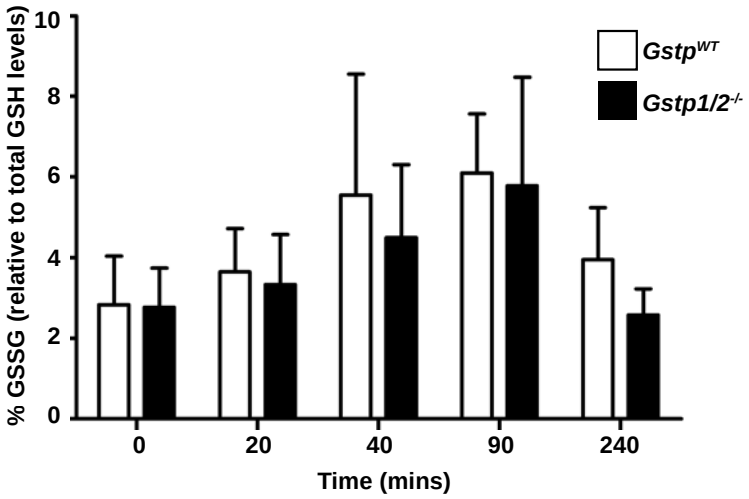
Supplementary Figure 1. HO-1 densitometry in *Gstp1*^{WT} and *Gstp1/2*$^{-/-}$ mice after acetaminophen administration.

The graph provides densitometric analysis of HO-1 Western blot results as described in Figure 1C of the main text. Whole cell liver lysates (10µg) were prepared from *Gstp1*^{WT} (WT) and *Gstp1/2*$^{-/-}$ (KO) male mice after a single oral dose of APAP (300mg/kg) and harvested at the time points shown. Lysates were analysed for HO-1 expression using Western blotting as described in Figure 1C of the main text. Densitometric analysis was performed using Multi Gauge V2.2 software (Fujifilm UK). HO-1 densitometry readings were normalised against GAPDH expression (n=3).



Supplementary Figure 2. JNK phosphorylation in *Gstp1*^{WT} and *Gstp1/2*$^{-/-}$ primary MEFs.

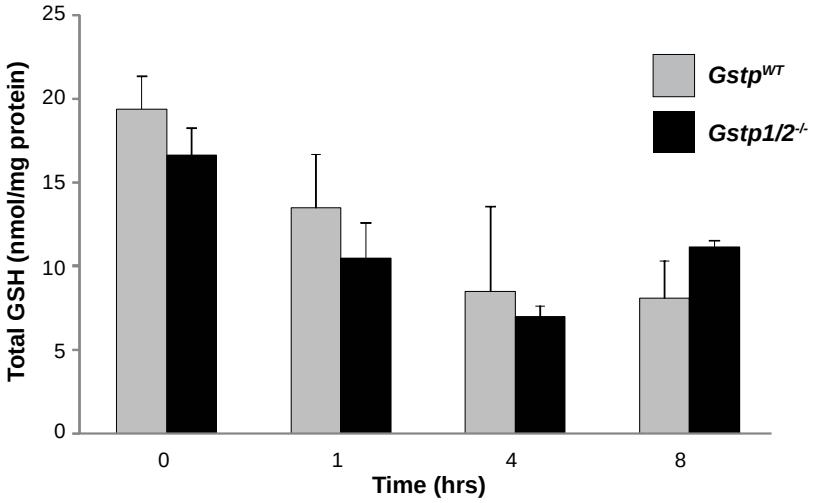
Primary mouse embryonic fibroblasts (MEFs) isolated from two independent cohorts (sets) of *Gstp1*^{WT} and *Gstp1/2*$^{-/-}$ mice were incubated with anisomycin at the doses described for 30 minutes. Lysates were then extracted and analysed (20µg lysate) by Western blotting.



Supplementary Figure 3. Oxidised glutathione levels in *Gstp1*^{WT} and *Gstp1/2*$^{-/-}$ mice in response to acetaminophen.

Male *Gstp1*^{WT} and *Gstp1/2*$^{-/-}$ mice were administered a single oral dose of acetaminophen (APAP; 300mg/kg) and harvested at time points indicated. Livers were removed, washed in phosphate-buffered saline and analysed for oxidised glutathione levels as detailed in *'Materials and Methods'* (n=8). Error bars show mean ± standard deviation.

A



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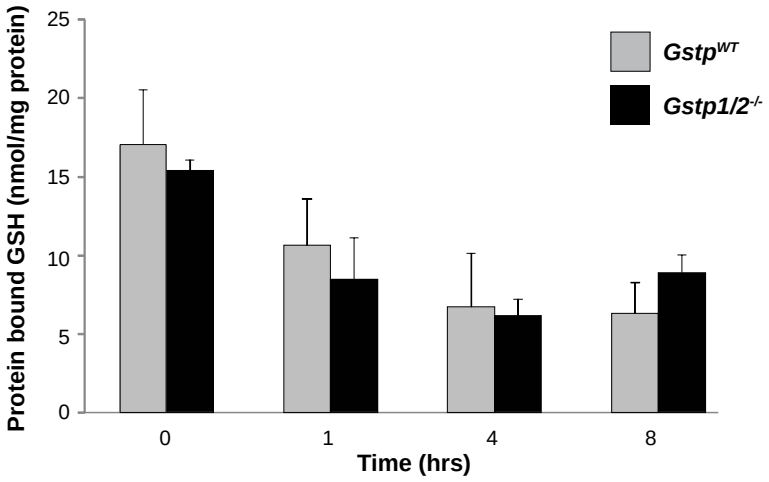

Supplementary Figure 4. Glutathione levels and protein S-glutathionylation in *Gstp1^WT^* and *Gstp1/2^-/-^* mice in response to buthionine sulfoximine.

Male *Gstp1^WT^* and *Gstp1/2^-/-^* mice were administered a single oral dose of buthionine sulfoximine (BSO; 0.9g/kg) and harvested at time points indicated. Livers were removed, washed in phospho-buffered saline and analysed for total glutathione levels **(A)** and total levels of protein S-glutathionylation **(B)** as detailed in *'Materials and Methods'*. Error bars show mean ± standard deviation.

Supplementary Table 1. nLC-MS/MS analysis of proteins identified as S-glutathionylated after acetaminophen treatment in *Gstp1*^{WT} and *Gstp1/2*$^{-/-}$ mice.

S-glutathionylated proteins were isolated from *Gstp1*^{WT} and *Gstp1/2*$^{-/-}$ mice after a single dose of acetaminophen (APAP; 300mg/kg, 40 minutes) as described in Figure 4 and subjected to nLC-MS/MS analysis. Parameters for MS analysis are provided in the Table and under *'Materials and Methods'*.

Supplementary Table 2. Pathway and Process analysis of proteins specifically S-glutathionylated in *Gstp1/2*$^{-/-}$ mice, 40 minutes after acetaminophen treatment.

The table shows Metacore software analysis of enriched pathways and processes from proteins specifically S-glutathionylated in *Gstp1/2*$^{-/-}$ mice after APAP treatment (see Figure 4a). The 'adjusted p value' stated corrects for multiple testing using the Benjamini-Hocberg correction method as described under *'Materials and Methods'*. Pathways with an adjusted p value of less than 0.05 were deemed significant.