Supplementary Information

3-Mercaptopyruvate sulfurtransferase produces potential redox regulators

cysteine- and glutathione-persulfide (Cys-SSH and GSSH) together with

signaling molecules H_2S_2, H_2S_3 and H_2S

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a

b
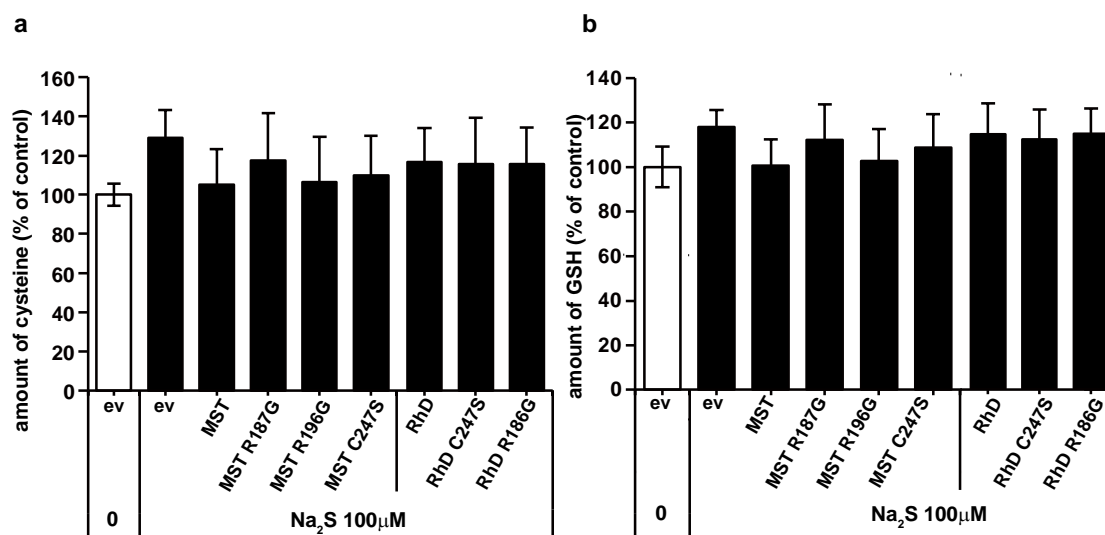
Supplementary Figure S1.

H_2S does not change cysteine and GSH levels. **a** and **b**. There is no significant difference in the levels of cysteine (**a**) and GSH (**b**) in cells expressing 3MST or rhodanese (RhD) and their mutants in the presence of 100 μM Na_2S. Monobromobimane adducts of cysteine and GSH were detected by LC with a fluorescence detector (n = 5). All data expressed as mean ± s.e.m.
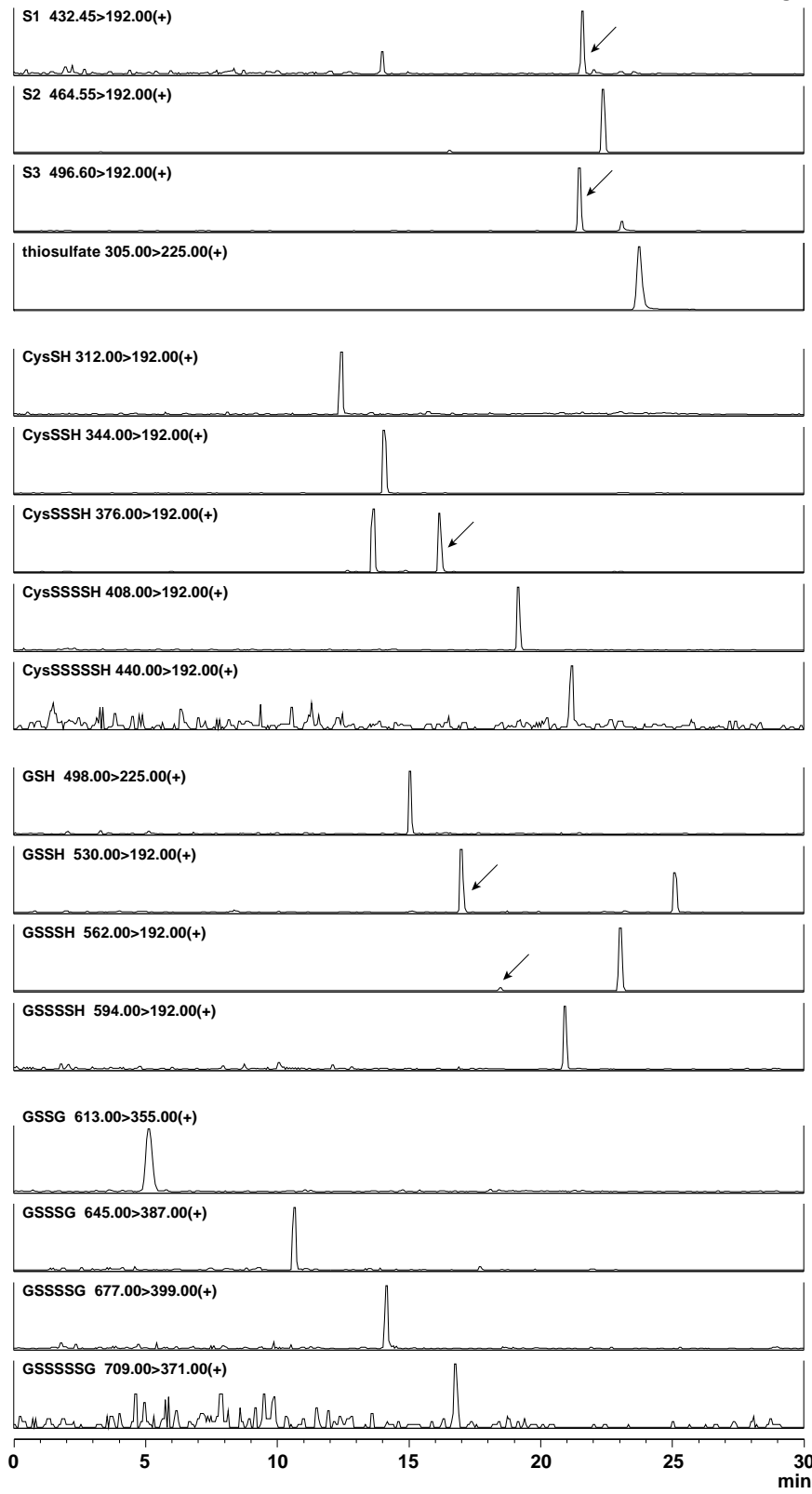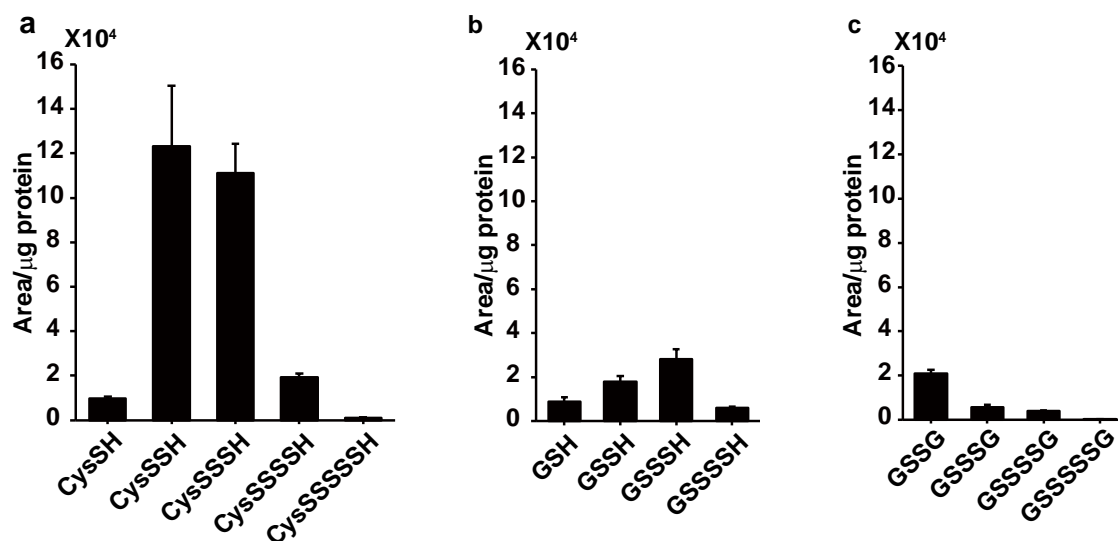
Supplementary Figure S2.

A representative chromatogram of LC-tandem mass spectrometry (LC-MS/MS). LC-MS/MS chromatograms are shown for monobromobimane adducts of S_1, S_2, S_3, thiosulfate, Cys-SH, Cys-SSH, Cys-SSSH, Cys-SSSSH, Cys-SSSSSH, GSH, GSSH, GSSSH, and GSSSSH. GSSG, GSSSG, GSSSSG, and GSSSSSG were also detected. Note that S_1, S_2 and S_3 are detected as di-bimane adducts, while others are mono-bimane adducts.
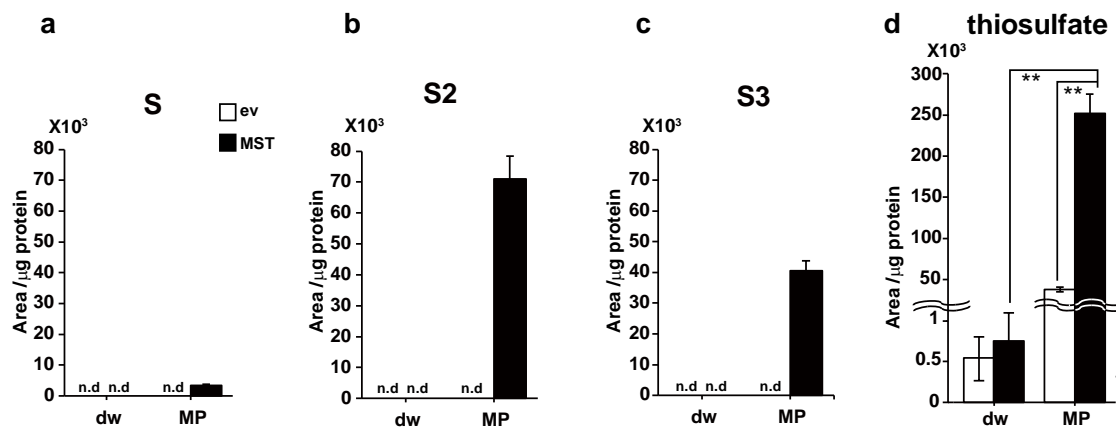
Supplementary Figure S3.

Comparison of the levels of Cys-SS$_n$H, GSS$_n$H and GSS$_n$G produced by 3MST. **a-c**.

The relative levels of Cys-SS$_n$H (**a**), GSS$_n$H (**b**), and GSS$_n$G (**c**) produced in lysates of

COS cells expressing 3MST in the presence of 100 μM 3MP (n = 3). Note that the

reaction mixture of lysates contained approximately 1 μM cysteine and 10 μM GSH.

Note that data were extracted from Fig. 3. All data expressed as mean ± s.e.m.

Supplementary Figure S4.

Production of H_2S, H_2S_2, H_2S_3 and thiosulfate in lysates of cells expressing 3MST.

a-d. H_2S (**a**), H_2S_2 (**b**), H_2S_3 (**c**) and thiosulfate (**d**) were produced in lysates of cells expressing 3MST (filled bar) in the presence of 100 μM 3MP, but they were much less in those transfected with an empty vector (open bar) (n = 3). Note that the reaction mixture of lysates contained approximately 1 μM cysteine and 10 μM GSH.

** $p < 0.01$ by ANOVA. All data expressed as mean $\pm$ s.e.m. N. d.: Not detected.
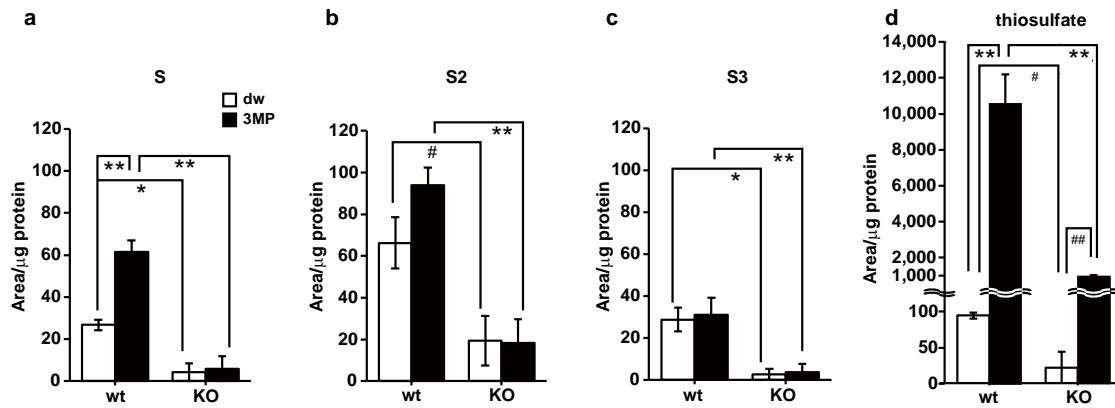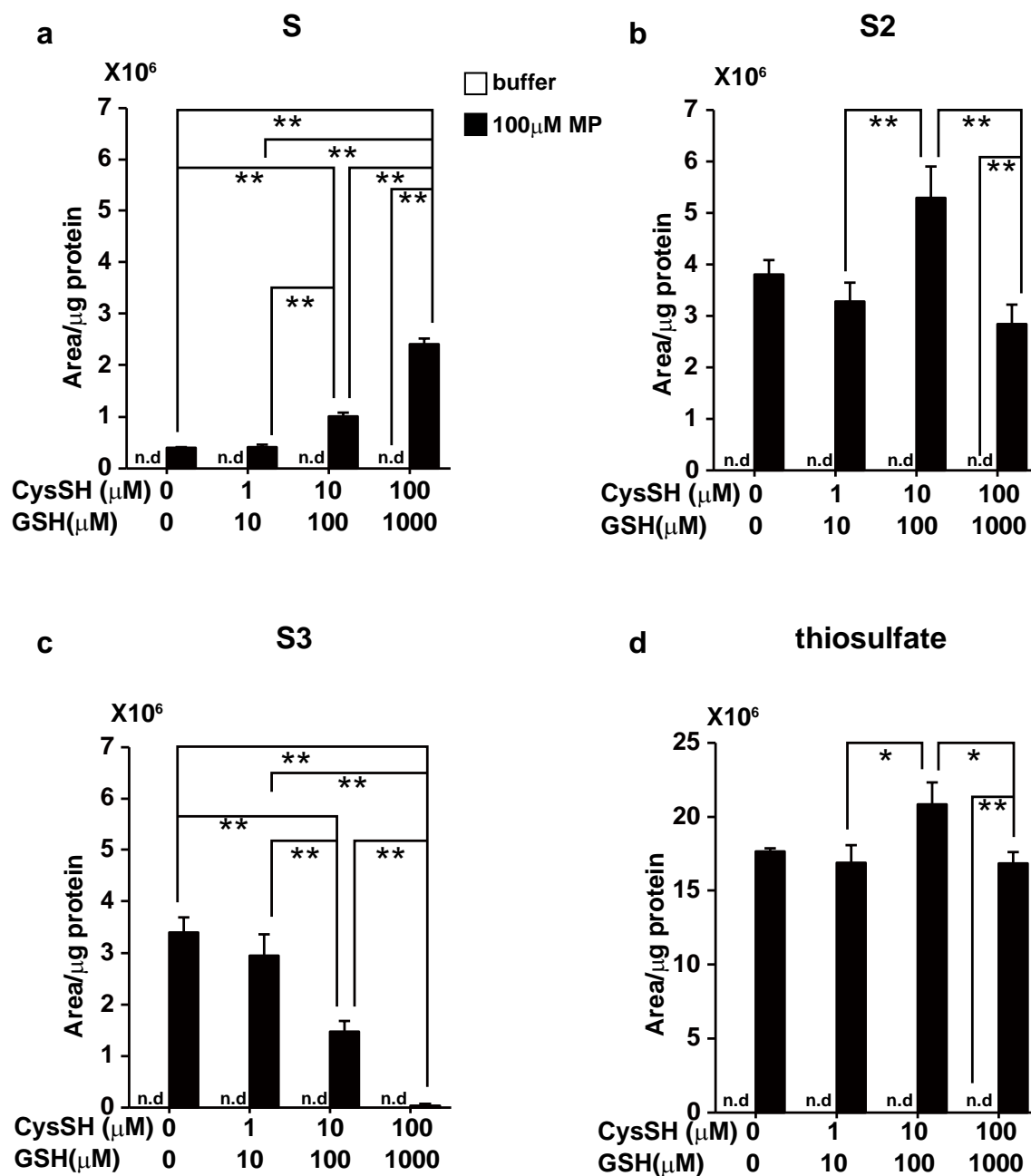
Supplementary Figure S5.

Production of H_2S, H_2S_2, H_2S_3 and thiosulfate in whole cells. **a-d**. Production of H_2S

(**a**), H_2S_2 (**b**), H_2S_3 (**c**) and thiosulfate (**d**) in brain cell suspension prepared from the

wild-type (wt) mice and 3MST-KO (KO) were shown after cells were exposed to 500

μM 3MP (filled bar) or medium without 3MP (open bar) (n = 3 for wt, n = 5 for KO).

Note that approximately 10% of 3MP was incorporated into cells 15 min after

exposure to 3MP and metabolized by 3MST [14]. ** $p < 0.01$, * $p < 0.05$ by ANOVA. # p

< 0.05 by Student t-test. All data expressed as mean ± s.e.m.

a S

b S2

c S3

d thiosulfate

Supplementary Figure S6.

Production of H2S, H2S2, H2S3 and thiosulfate by recombinant 3MST in the presence

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of various concentrations of cysteine and GSH. **a-d**. The levels of H_2S (**a**), H_2S_2 (**b**), H_2S_3 (**c**) and thiosulfate (**d**) by recombinant 3MST with 100 μM 3MP in the presence of indicated concentrations of cysteine and GSH. (n = 3) ** $p < 0.01$, * $p < 0.05$ by ANOVA. All data expressed as mean $\pm$ s.e.m. N. d.: Not detected.