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Supplementary Materials for

H_2S Signals Through Protein S-Sulfhydration

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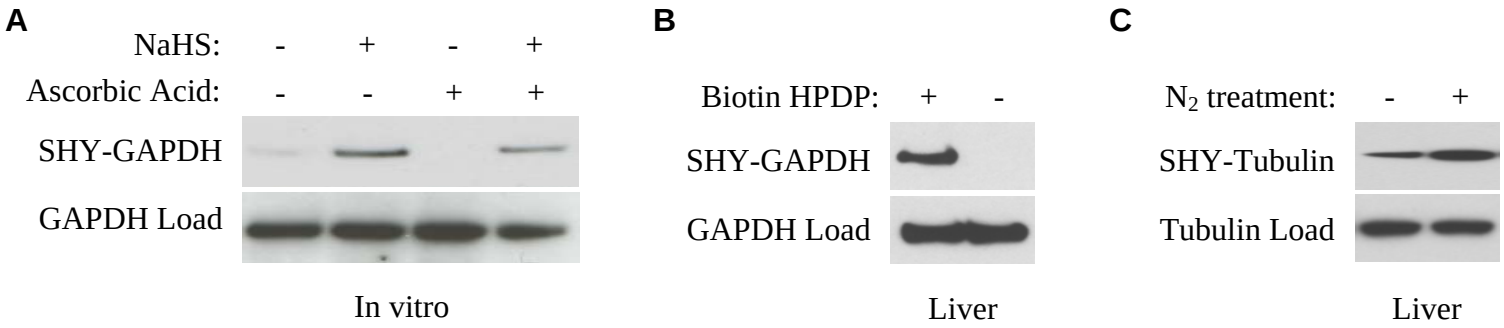

Fig. S1. Modified biotin switch assay for protein *S*-sulfhydration. (A) In vitro sulfhydration of purified GAPDH with NaHS with and without 1 mM ascorbic acid. The signal using an anti-GAPDH antibody is equally strong with and without ascorbic acid. **(B)** In vivo sulfhydration of GAPDH in liver with or without 4 mM biotin-HPDP. There was no signal without biotin-HPDP, indicating that the basal signal was not from endogenous biotinylation of the protein. **(C)** In vivo sulfhydration of β-tubulin in liver using assay buffers deoxygenated with nitrogen gas. Deoxygenated condition enhances the basal sulfhydration signal.

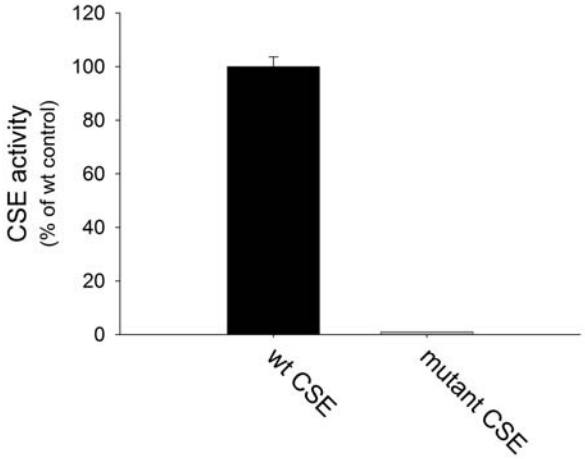

Fig. S2. The non–PLP-binding CSE mutant is catalytically inactive. CSE enzyme activity in vitro with wild-type and mutant CSE (L90/Y113/N160/D186/S208/K211 – all mutated to glycine) was assessed. The mutant enzyme was totally inactive.

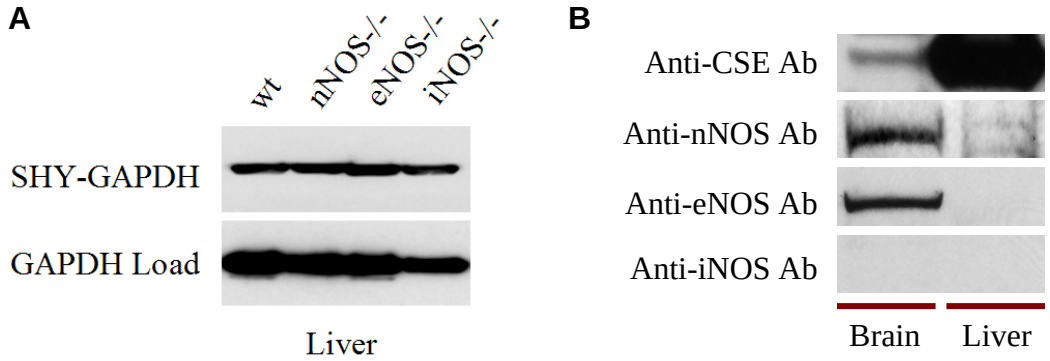

Fig. S3. The modified biotin switch assay is specific for sulfhydration. (A) Modified biotin switch assay for GAPDH in wild-type and *NOS*$^{-/-}$ liver lysates. *NOS*$^{-/-}$ samples show similar sulfhydration signal compared to wild-type. **(B)** CSE and NOS abundance in brain and liver. CSE is highly abundant in the liver whereas all the NOS enzymes are present primarily in the brain.

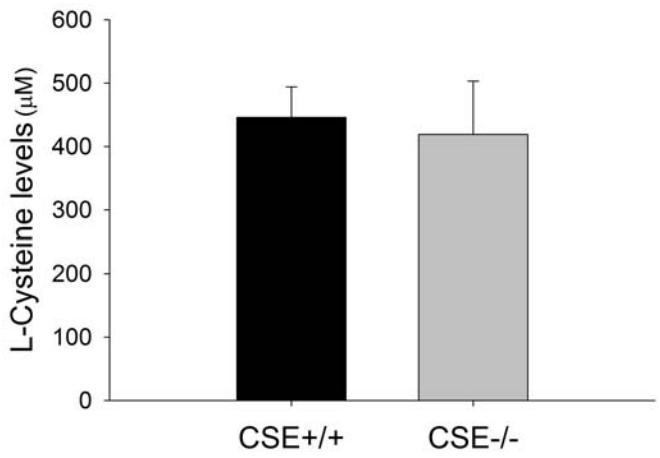

Fig. S4. L-Cysteine levels are comparable in wild-type and *CSE*$^{-/-}$ livers. Basal L-cysteine concentrations measured by HPLC are similar in wild-type and *CSE*$^{-/-}$ livers.

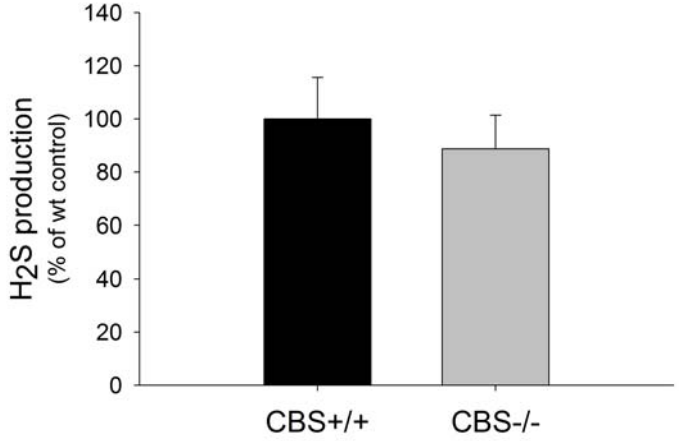

Fig. S5. Wild-type and *CBS*$^{-/-}$ livers show similar H_2S production. Measurements of H_2S production indicate that *CBS*$^{-/-}$ liver generates similar amounts of H_2S as its wild-type counterpart.

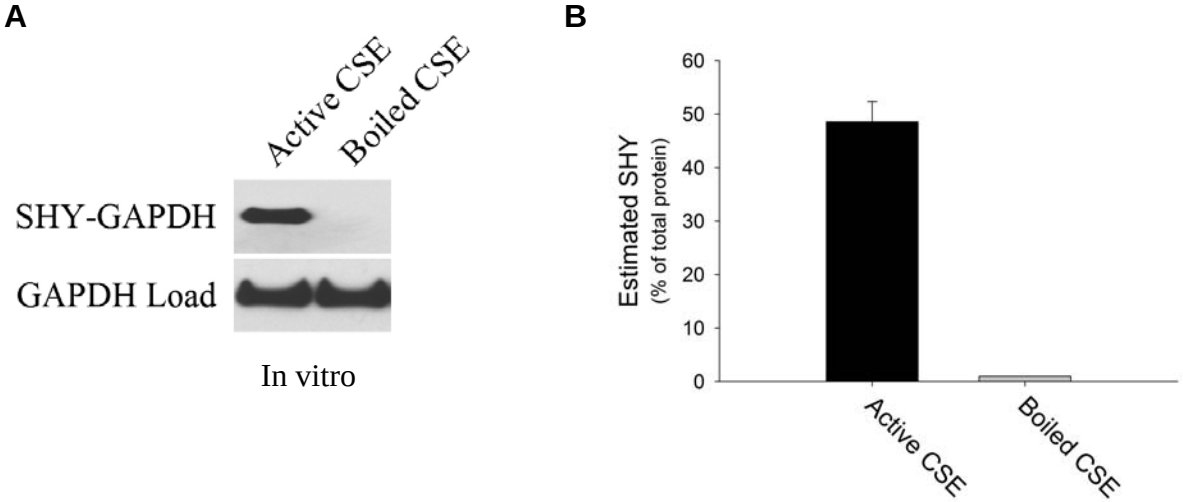

Fig. S6. GAPDH is substantially sulfhydrated during cell-free catalysis by CSE and L-cysteine. (A) Pure full-length human GAPDH protein (0.3 µg) is sulfhydrated within 30 min at 37°C during cell-free catalysis by 2 µg CSE and 0.45 mM L-cysteine. Inactivating CSE by boiling for 5 min prevents sulfhydration. **(B)** Densitometric quantitation shows around 50% of GAPDH is sulfhydrated under the above conditions.

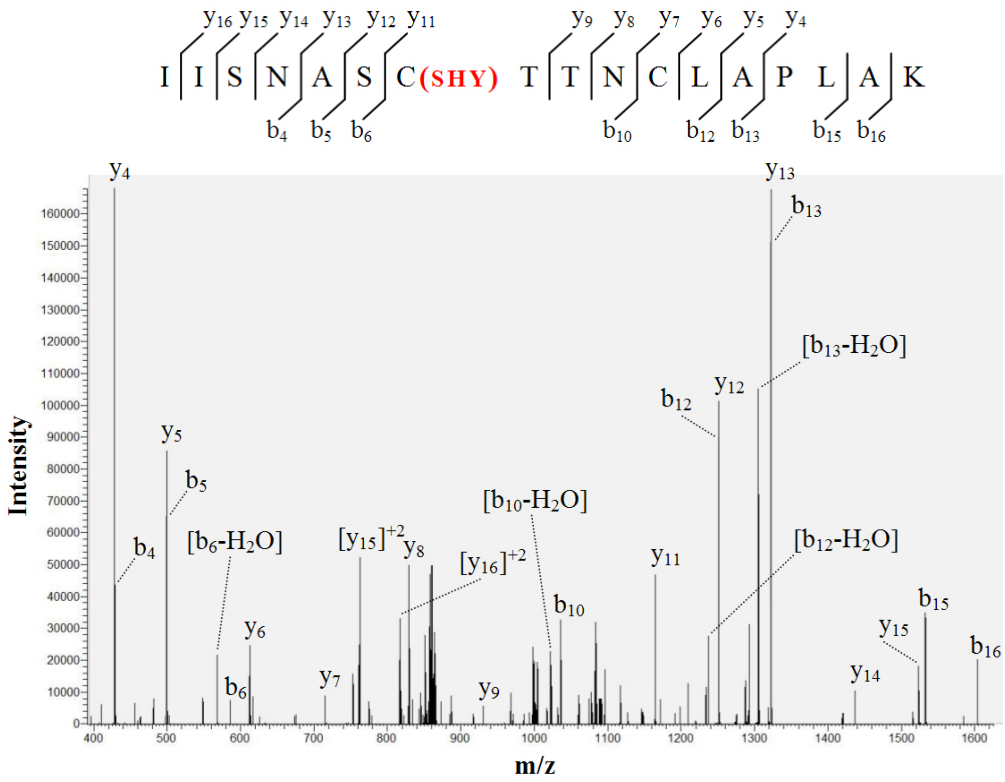

Fig. S7. GAPDH sulfhydration at Cys^{150} with LC-MS/MS. MS2 spectrum of purified wild-type human GAPDH protein treated with 100 µM NaHS for 30 min at 37°C shows sulfhydration of Cys^{150}.

	K_{ATP} SHY	
NaHS	+	+
DTT	–	+
% SHY	5.1± 0.25	0.2 ± 0.1

HEK293

Fig. S8. H_2S sulfhydrates ATP-sensitive potassium channels in HEK293 cells. The exogenously expressed Kir6.1 subunit of the ATP-sensitive potassium channel is sulfhydrated in HEK293 cells treated with 100 µM NaHS for 30 min at 37°C. The channel is not basally sulfhydrated in these cells, which lack the capacity to produce H_2S endogenously. Treatment with DTT (200 µM) for 10 min reverses the NaHS-mediated K_{ATP} sulfhydration.