Supplementary Information

Cystathionine γ-Lyase Self-Inactivates by Polysulfidation during Cystine Metabolism

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SUPPLEMENTARY METHODS

Plasmid construction

The rat CSE mutant C69V, C83V, C108V, C136V, C171V, C205V, C207V, C251V, C254V, C255V, C306V, and C309V was subcloned into pGEX-6P vector. The nucleotide sequence of each mutant was confirmed by DNA sequencing.

SUPPLEMENTARY FIGURE LEGENDS

Figure S1. CSE β-lyase activities of recombinant CSEs. (A) CSE β-lyase activities toward β-CA (3–10 mM) of wild-type and indicated mutants were measured by coupling a color enzymatic reaction with pyruvate oxidase and peroxidase as described in the Materials and Methods section. (B) CSE β-lyase activities toward cystine (0.1-1 mM) examined for the Cys-SSH level with a sulfane sulfur-specific fluorescent probe, SSP4, as described in the Materials and Methods section. The representative data were shown of three-four experiments.

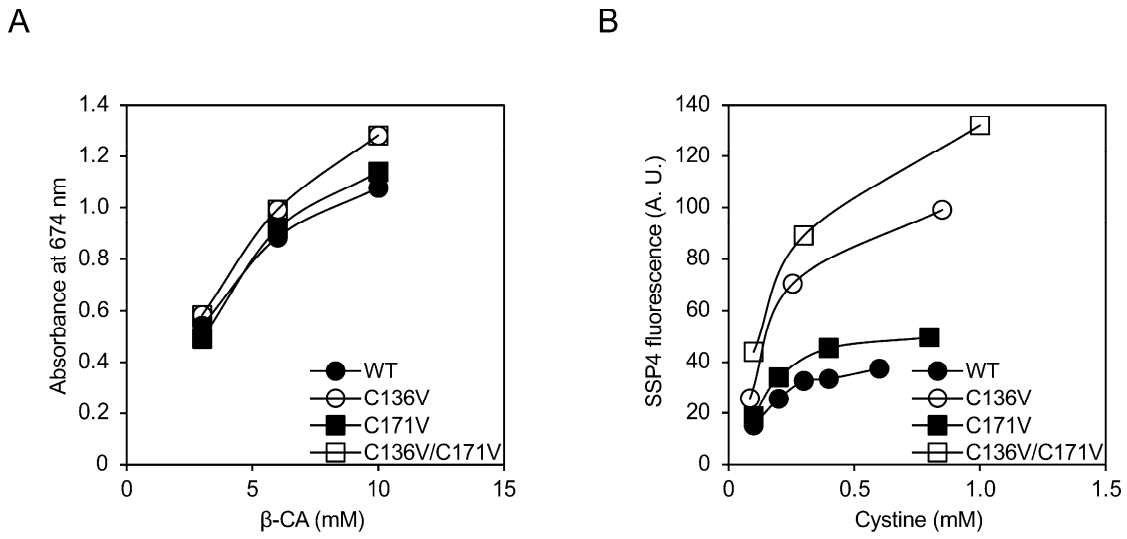

Figure S2. The cysteine residue that is sensitive to autoinactivation of CSE β-lyase activity toward cystine to generate Cys-SSH. (A) Purified recombinant CSEs preparation used in this study. Equal amounts (1 μg) of GST-cleaved wild-type CSE (WT) and the indicated mutants in E. coli cells were separated by 10% SDS-PAGE and stained by Coomassie Brilliant Blue. (B) CSE β-lyase reactions were examined for the Cys-SSH level with a sulfane sulfur-specific fluorescent probe, SSP4, as described in the Materials and Methods section. Values represent the mean ± S.E. for four

independent experiments. ****P < 0.0001 and ***P < 0.001 when compared with wild-type CSE.