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Supplementary Materials and Methods

Measurements of free amino acids in serum

The amino acids in serum (as well as homophenylalanine as the internal standard and homoarginine for the injection check) were derivatized using NBD-F in accordance with the manufacturer's instructions. The samples (10 μ l) were injected onto a reversed-phase C18 column (CAPCELL PAK C₁₈ MGII S5; 5.0 μ m, 3.0 \times 150 mm; Shiseido, Tokyo, Japan) for the separation of fluorescent-derivatized amino acids. The mobile phase consisted of elution solution A (10 mM citrate buffer [pH 6.2] containing 75 mM NaClO₄) and B (50% [v/v] acetonitrile). A gradient of increasing solution B as follows was used: 5–10% for 20 min, 10–37% for 35 min, 37–38% for 5 min, 38–79.3% for 10 min, 79.3–90% for 5 min, and 100% for 5 min. All gradient steps were controlled by a LaChrom Elite chromatography workstation (Hitachi, Tokyo, Japan), and peaks in fluorescence (Ex. 480 nm; Em. 530 nm) were measured using an L-2400 fluorescence detector (Hitachi). All amino acids were identified based on their retention times and concentrations were calculated relative to the calibrated standard solutions (Wako, Osaka, Japan).

Measurements of free thiol compounds in serum

Total cysteine/homocysteine (Hcy)/glutathione (GSH)/cysteinylglycine (CG)/ γ -glutamylcysteine (γ Glu-Cys), which gives rise to their respective thiol compounds after reductive cleavage of disulfide bonds [8], were measured by the following procedure. The serum samples or standard reagents (20 μ l) were mixed with 10 μ l of phosphate-buffered saline (pH 7.4), 10 μ l of 80 μ M *N*-(2-mercaptopropionyl)glycine (MPG; internal standard), and 4 μ l of 0.349 M (100 g/l) Tris(2-carboxyethyl)phosphine hydrochloride, and then incubated at room temperature for 30 min. Then the mixtures were added to 36 μ L of 0.612 M (100 g/l) trichloroacetic acid (TCA)/1 mM EDTA (pH 8.0) solution and centrifuged at 13,000 *g* at 20°C for 10 min. The 50 μ l supernatant was mixed with 185 μ l of a mixture of 50 μ g SBD-F in 175 μ l of 125 mM borate-HCl (pH 9.5)/4.0 mM EDTA (pH 8.0) and 10 μ l of 1.55 M NaOH, and incubated at 60°C for 1 h. The samples (10 μ l) were injected onto the same C18 column using an Agilent 1100 series chromatography workstation (Agilent, Santa Clara, CA, USA) for the separation of fluorescent-derivatized thiol-containing amino acids. The mobile phase consisted of elution solution A (0.1 M sodium acetate [pH 4.5]) and B (50% [v/v] methanol). A gradient of increasing solution B as follows was used: 6% for 5 min and 6–100% for 10 min. The peaks in fluorescence (Ex. 385 nm; Em. 515 nm) were measured using an Agilent 1260 fluorescence detector. All thiol compounds were identified based on their retention times and concentrations were calculated relative to the calibrated standard solutions (Wako).

Measurements of tryptophan in serum

Tryptophan was independently quantified by its own fluorescence without NBD-F derivatization. The samples (10 μ l) were injected onto the same C18 column using an Agilent 1100 series chromatography workstation. The mobile phase consisted of elution solution A (0.1 M sodium acetate [pH 4.5]) and B (50% [v/v] methanol). A gradient of increasing solution B as follows was used: 5–7.2% for 13 min. The peaks in fluorescence (Ex. 295 nm; Em. 340 nm) were measured using an Agilent 1260 fluorescence detector.

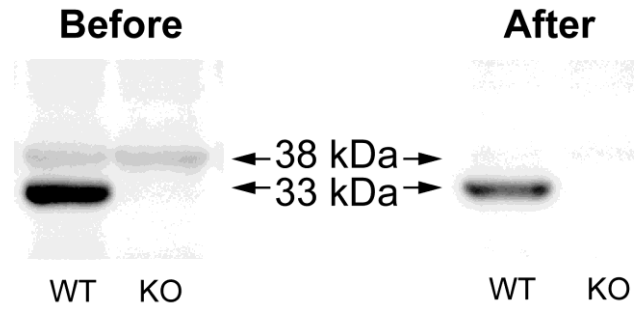
Supplementary Figure 1. Mouse *Mpst* gene and CRISPR/Cas9-mediated deletion of exon 2.

A. Mouse *Mpst* gene (total 7,304 bp)

1 100

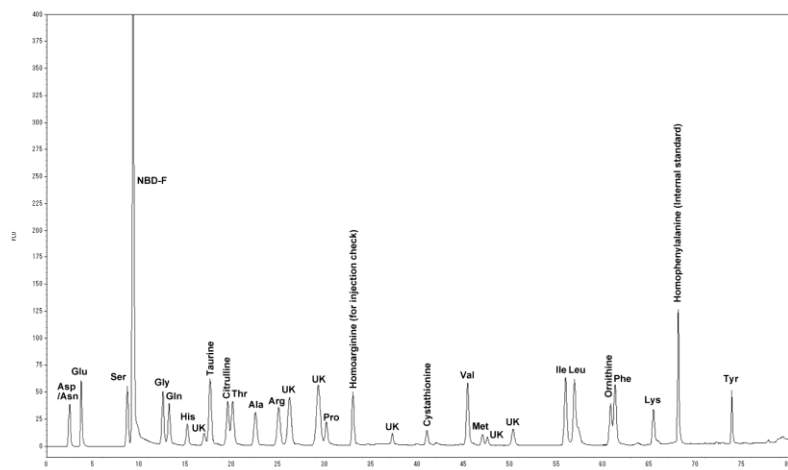
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Exon, CDS, Intron, Exon1 variation, **CRISPR-Cleavage site**

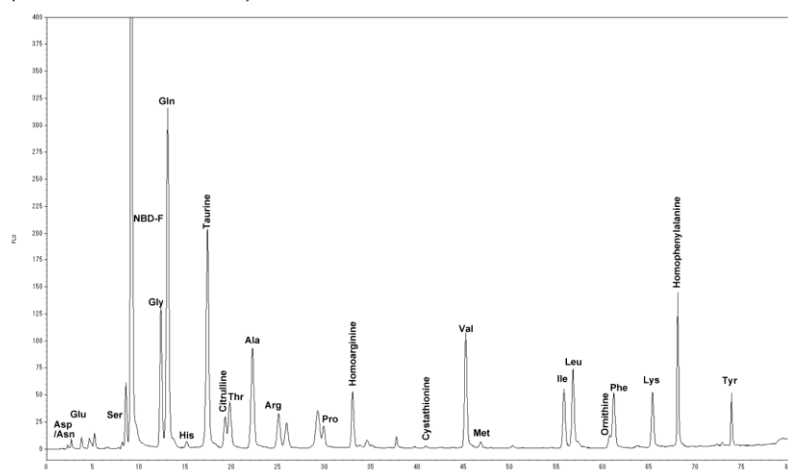


Supplementary Figure 2. Pre-incubation of anti-mouse Mpst rabbit polyclonal antibody with recombinant mouse Tst proteins abolishes 38-kDa (Tst) but not 33-kDa (Mpst) bands. His-tagged mouse Tst recombinant proteins were pre-incubated with anti-mouse Mpst antiserum in PBS at 4°C overnight, and the mixture was passed through the TALON Metal Affinity Resins to remove Tst (not Mpst)-associated antibody. Hepatic Mpst and Tst proteins in WT and Mpst(1st)-KO mice were detected with anti-mouse Mpst antibody (Before) or that pre-incubated with Tst recombinant proteins (After).

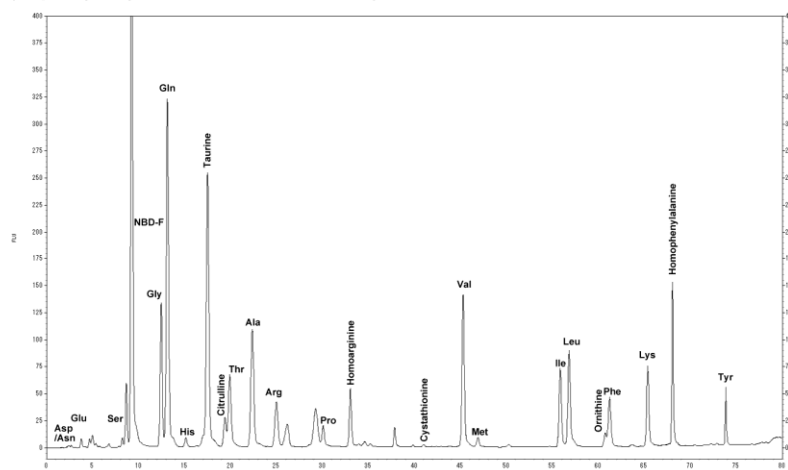
A (Standard reagents)



B (WT mouse serum)

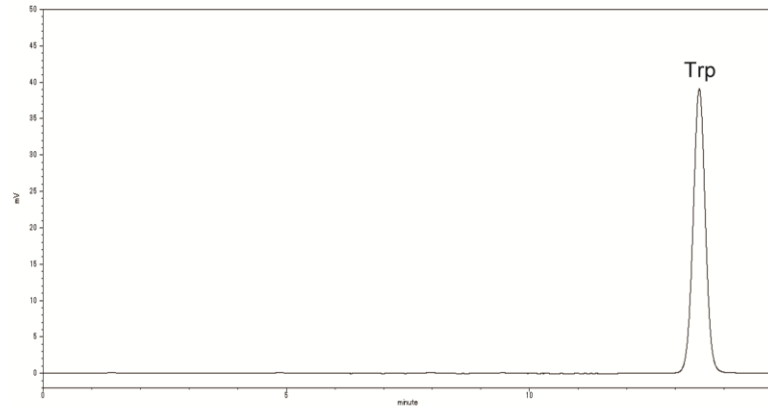


C (Mpst(1st)-KO mouse serum)

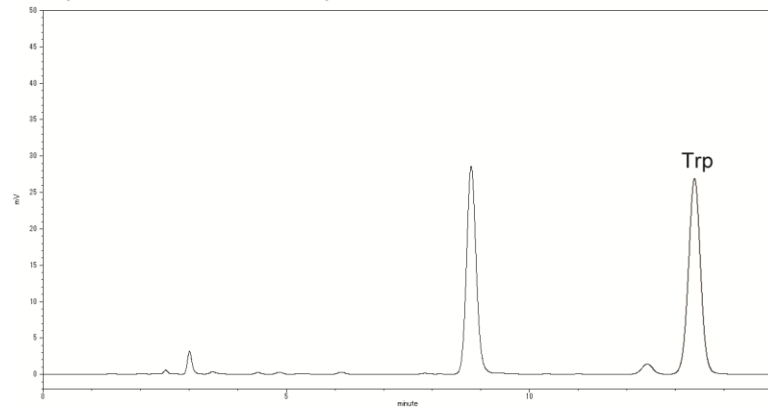


Supplementary Figure 3. Representative chromatograms in the measurements of free amino acids in serum samples. Standard amino acid reagents (A), WT mouse serum (B), and Mpst (1st)-KO mouse serum (C), were analyzed by NBD-F labeling and HPLC coupled with a fluorescence detector. UK, unknown. Homophenylalanine peaks were used as the internal standard and homoarginine peaks for the injection check. The concentrations of the standard reagents used in (A) are 250 μ M for cystathionine and 125 μ M for other amino acids. Most unknown peaks seem to be derived from the degradation products of unreacted NBD-F.

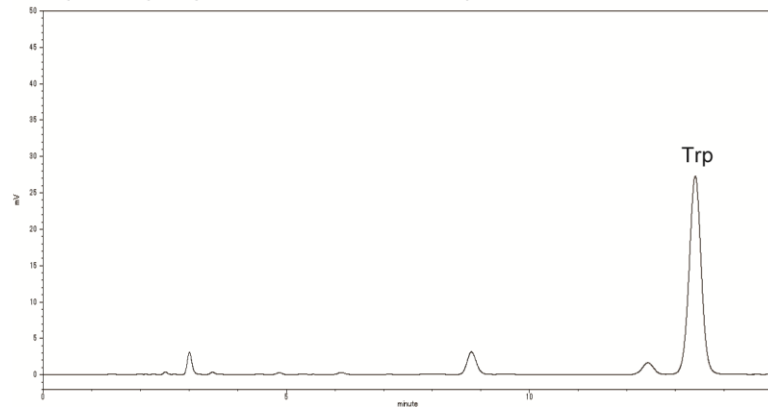
A (Standard reagents)



B (WT mouse serum)

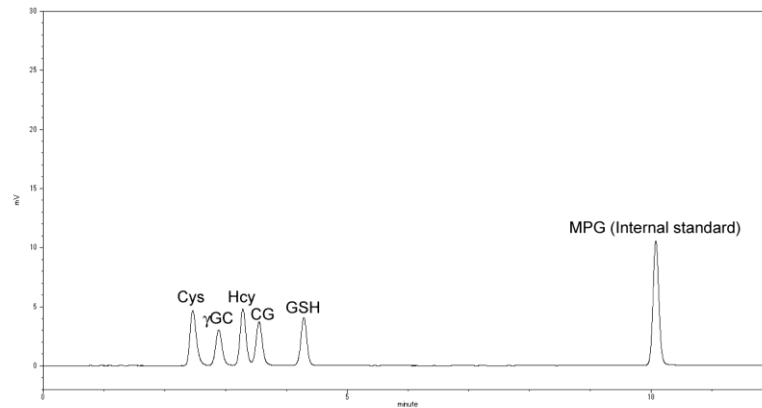


C (Mpst(1st)-KO mouse serum)

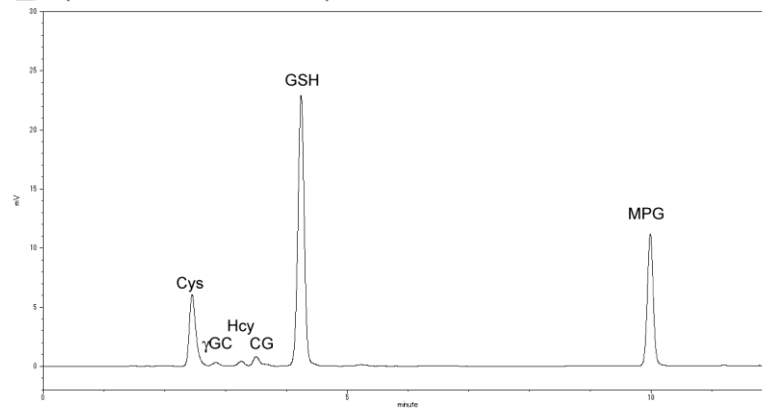


Supplementary Figure 4. Representative chromatograms in the measurements of tryptophan (Trp) in serum. Standard Trp reagent (A), WT mouse serum (B), and Mpst (1st)-KO mouse serum (C), were analyzed by HPLC coupled with a fluorescence detector. The concentration of the standard Trp reagent in (A) is 100 μ M.

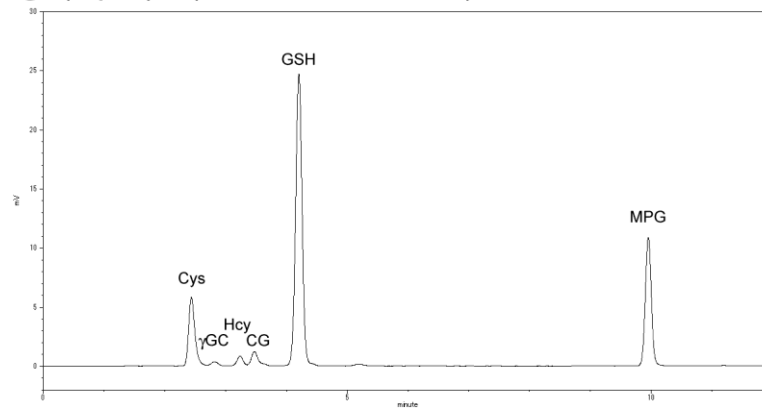
A (Standard reagents)



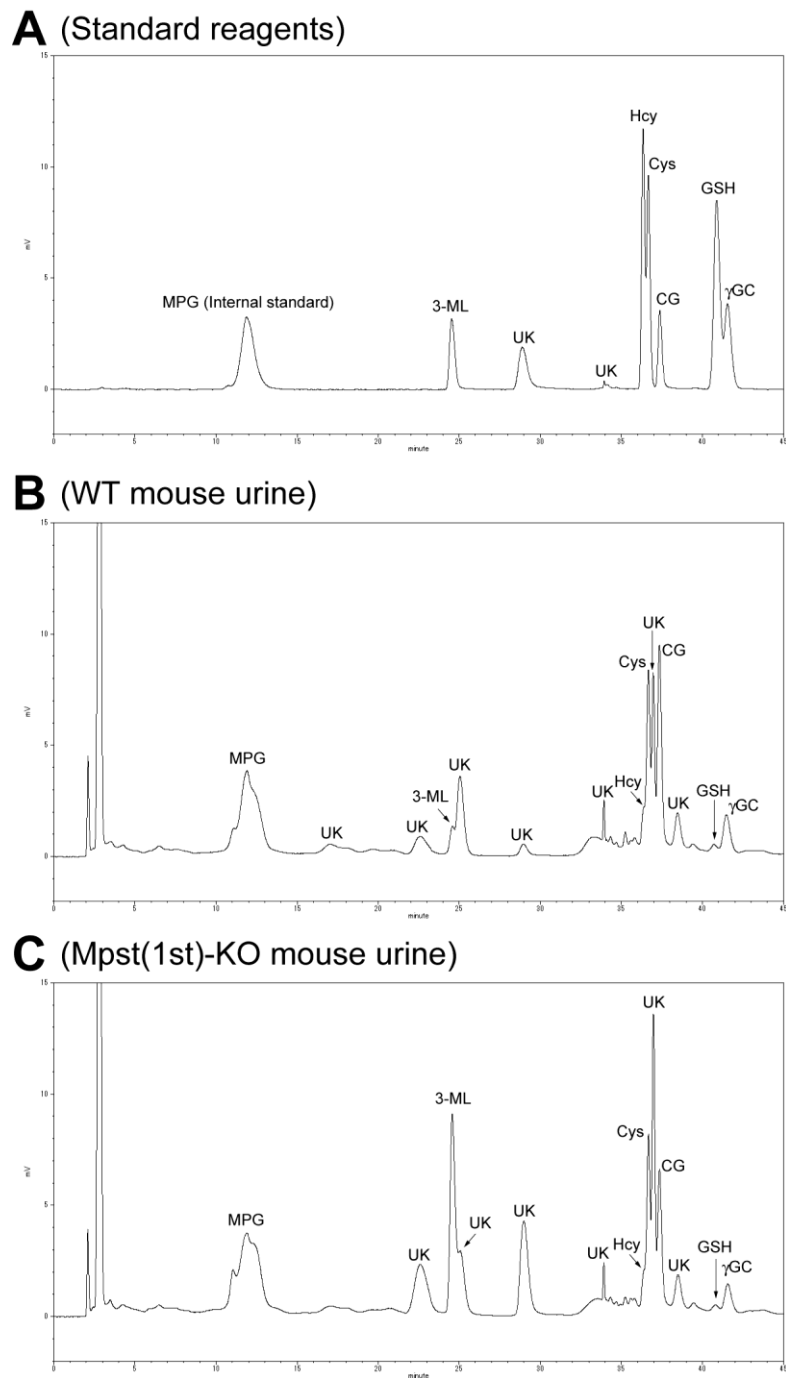
B (WT mouse serum)



C (Mpst(1st)-KO mouse serum)



Supplementary Figure 5. Representative chromatograms in the measurements of thiol compounds in serum samples. Standard thiol compounds (A), WT mouse serum (B), and Mpst (1st)-KO mouse serum (C), were analyzed by SBD-F labeling and HPLC coupled with a fluorescence detector. γ GC, (total) γ -Glu-Cys; CG, (total) Cys-Gly; Hcy, (total) homocysteine; GSH, (total) glutathione; MPG, *N*-(2-mercaptopropionyl)glycine (as the internal standard). The concentrations of the standard reagents in (A) are 200 μ M for Cys, 100 μ M for γ GC, 40 μ M for Hcy, 20 μ M for CG and 40 μ M for MPG.



Supplementary Figure 6. Representative chromatograms for measurements of 3-mercaptolactate (3-ML) in serum. Standard thiol compounds (A), WT mouse urine (B), and Mpst (1st)-KO mouse urine (C), were analyzed by SBD-F labeling and HPLC coupled with a fluorescence detector. UK, unknown; 3-ML, 3-mercaptolactate; γ GC, (total) γ -Glu-Cys; CG, (total) Cys-Gly; Hcy, (total) homocysteine; GSH, (total) glutathione; MPG, *N*-(2-mercaptopropionyl)glycine (as the internal standard). The concentrations of the standard reagents in (A) are 40 μ M for MPG, 100 μ M for 3-ML, 200 μ M for Hcy, 1 mM for Cys, 40 μ M for CG, 400 μ M for GSH, and 200 μ M for γ GC.