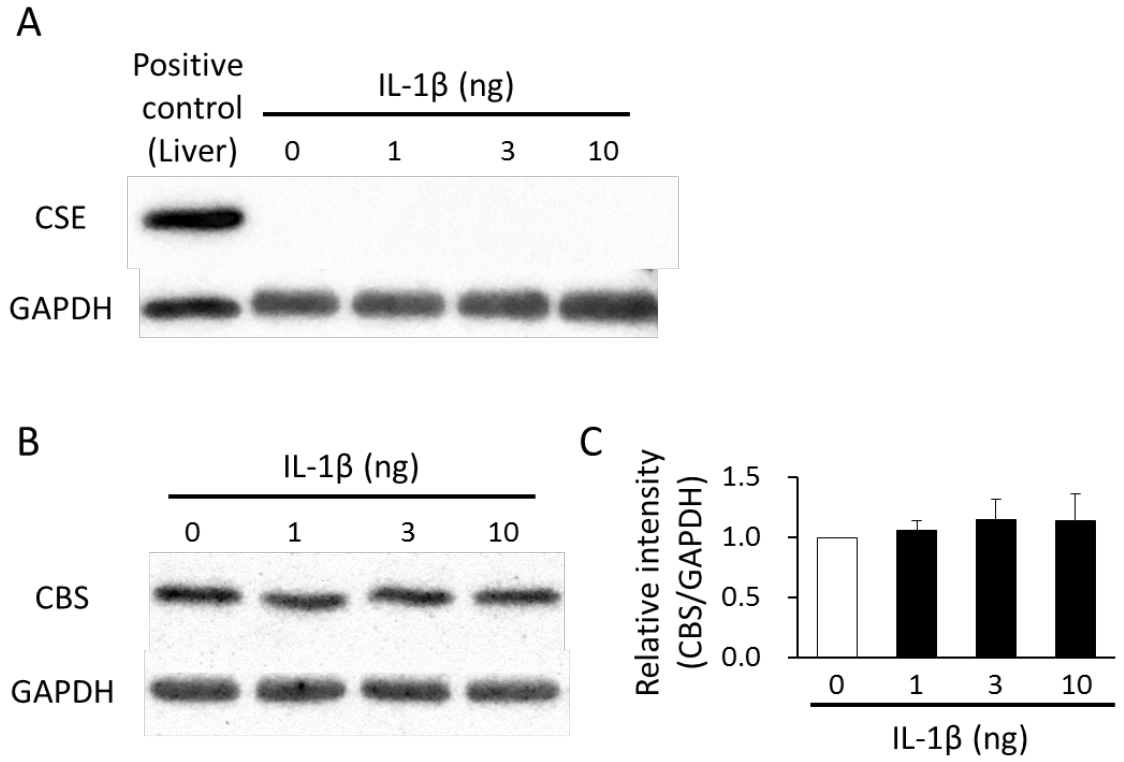
Supplemental Method

Western blot

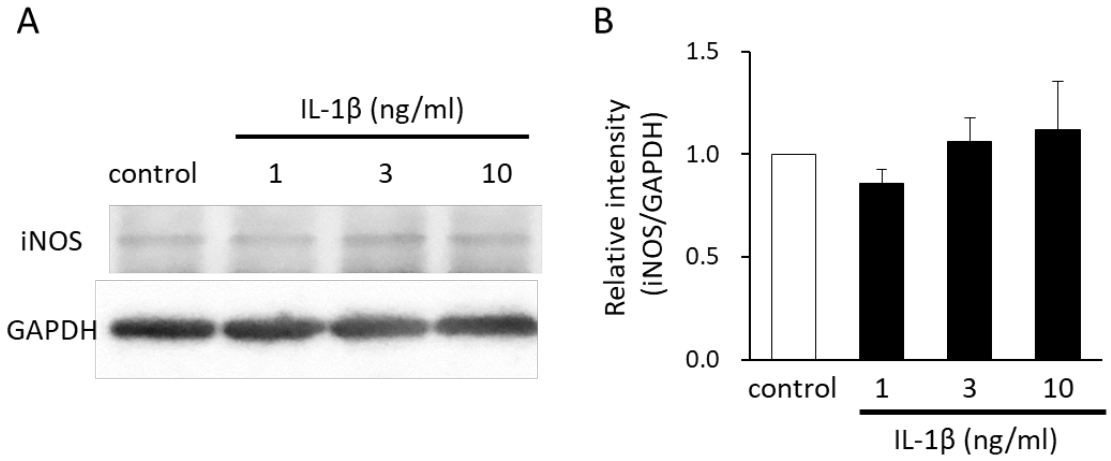
Cells were lysed in radio immune precipitation assay buffer (20 mM Tri-HCl, 150 mM NaCl, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate, and 1% Triton X-100, pH 7.5) containing Protease Inhibitor Cocktail (Sigma-Aldrich/Merck). After mechanical homogenization and sonication, the lysates were centrifuged at 10,000 g for 10 min at 4°C, and the supernatants were collected. Protein concentrations of lysates were determined by using the RC DC Protein Assay Kit (Bio-Rad Laboratories, Hercules, CA, USA). After denaturation at 95°C for 5 min in loading buffer (50 mM Tris-HCl, 2% sodium dodecyl sulfate, 12.5% glycerol, 0.01% bromophenol blue, and 100 mM DTT, pH 6.8), equal amounts of the samples were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride membranes. The membranes were blocked with 5% skim milk in Tris buffered saline (20 mM Tris-HCl, 137 mM NaCl, pH 7.6) containing 0.1% Tween-20 (TBST) for 30 min at 24 ± 3°C. They were then incubated with anti-CSE (1 : 2000, Protein Tech, Manchester, UK), anti-CBS (1 : 3000, Protein Tech), anti-iNOS (1 : 1000, Merck Millipore, Darmstadt, Germany) or peroxidase-conjugated anti-glyceraldehyde 3-phospahte dehydrogenase (GAPDH) antibodies (1 : 20000, Sigma-Aldrich/Merck) in TBST containing skim milk overnight at 4°C. The membranes were incubated with horseradish peroxidase-conjugated secondary antibodies (1 : 2000 for CSE and 1 : 3000 for CBS) in 1% skim milk in TBST for 1 h at 24 ± 3°C, and antibody binding was visualized by ECL Prime (GE Healthcare, Little Chalfont, UK) in Lumicube (Liponics, Tokyo, Japan).Band intensities were measured with software (Image J, National Institutes of Health, Bethesda, MD, USA).

Supplemental Figure



Suppl. Fig. 1　CSE and CBS expression in IL-1ß-treated cells

(A, B) Representative western blot of CSE (A) and CBS (B) in non-treated or IL-1ß (1-10 ng/ml, 24 h)-treated cells. (C) Relative band intensities of CBS normalized to GAPDH (n = 4).



Suppl. Fig. 2　iNOS expression in IL-1ß-treated cells

(A) Representative western blot of iNOS in non-treated or IL-1ß (1-10 ng/ml, 24 h)-treated cells. (B) Relative band intensities of iNOS normalized to GAPDH (n = 4).