

**Supporting Information**

**Hydrosulfide-methemoglobin-albumin cluster: A hydrogen sulfide donor**

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## **Materials and Methods**

### ***Measurement of physicochemical characteristics***

Diameter and polydispersity index (PD-index) were measured using a dynamic light scattering (DLS) instrument (Mobius, Wyatt Technology Corp., Santa Barbara, CA, USA). The zeta-potential was determined using a zeta-potential analyzer (ELSZ2KOP, Otsuka Electronics, Osaka, Japan).

### ***Size-exclusion chromatography (SEC)***

SEC was performed using a YMC-Pack Diol-300 column (8.0 mm × 300 mm, 5 μm, YMC Co. Ltd., Kyoto, Japan) with 50 mM phosphate buffer (pH 7.4) as the mobile phase. The flow rate was 0.5 mL/min. UV wavelength was 280 nm.

### ***Circular dichroism (CD)***

CD spectra were measured at 0.2 μM (as metHb) from 260 to 200 nm using a spectropolarimeter (J-1100, JASCO Corp., Tokyo, Japan).

### ***Electrophoresis***

Native-PAGE was performed by loading samples onto a 6% native polyacrylamide gel (SuperSep Ace; FUJIFILM Wako Pure Chemical, Osaka, Japan). Gel was stained with Coomassie Blue R-250 (CBB Stain One Super, Nacalai Tesque, Kyoto, Japan), and images were recorded by an Amersham Imager 600 (Cytiva, Marlborough, MA, USA).

### ***Measurement of glutathione (GSH) and GSH-to-oxidized GSH (GSH/GSSG) in the liver***

The collected livers were homogenized, and GSH and GSH/GSSG in the liver were measured using GSSG/GSH Quantification Kit (Dojindo, Japan) according to manufactures instruction.

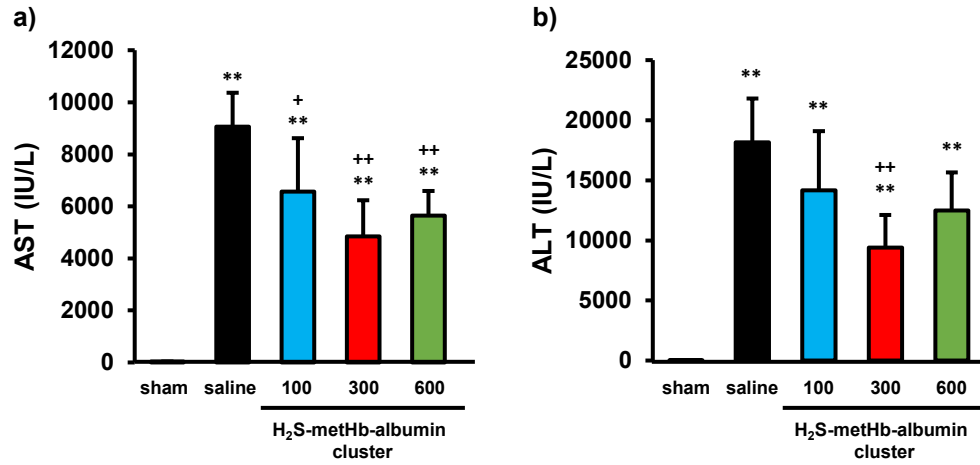
### ***Measurement of malondialdehyde (MDA) levels***

The collected livers were homogenized, and MDA levels were measured using TBARS Assay Kit (Cayman Chemical, Ann Arbor, MI, USA) according to manufactures instruction.

### ***Measurement of inflammatory cytokine***

Cytokines (IL-1 $\beta$ , IL-6, TNF- $\alpha$ , IFN- $\gamma$ ) in plasma were measured using Quantikine ELISA Kit (R&D Systems, Inc., Minneapolis, MN, USA) according to manufacturer's instruction.

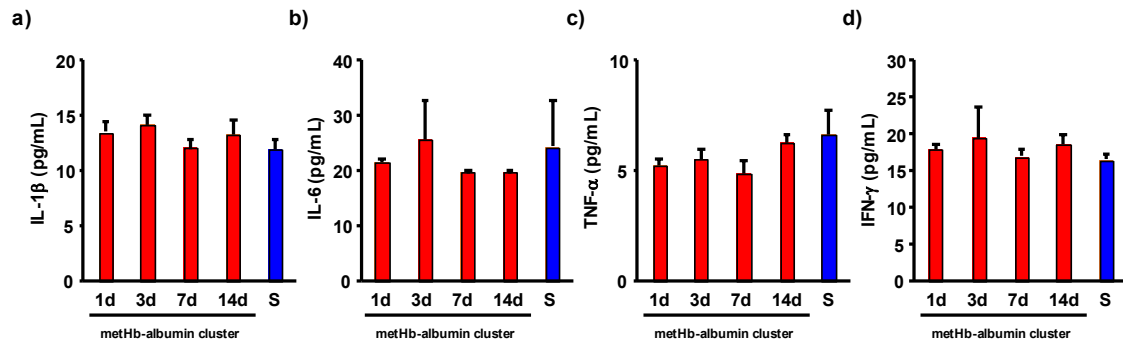
## Results



**Fig. S1. The dose-dependent therapeutic efficacy of H<sub>2</sub>S–metHb-albumin cluster against a mouse model of hepatic ischemia-reperfusion injury.**

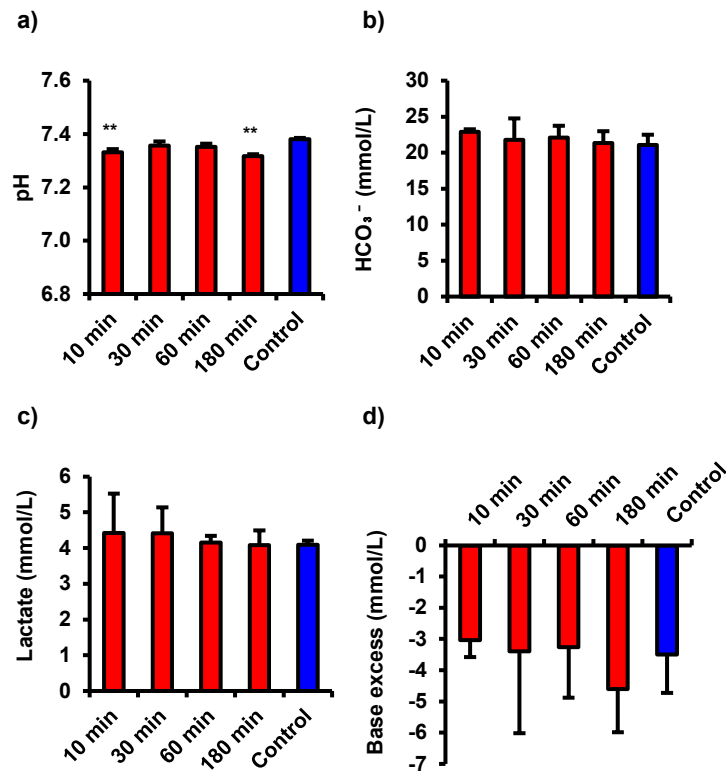
Plasma levels of **a)** aspartate aminotransferase (AST) and **b)** alanine aminotransferase (ALT).

n = 6 per group, mean + S.D. \*\**p* < 0.01 vs. sham, +*p* < 0.05, ++*p* < 0.01 vs. Saline.



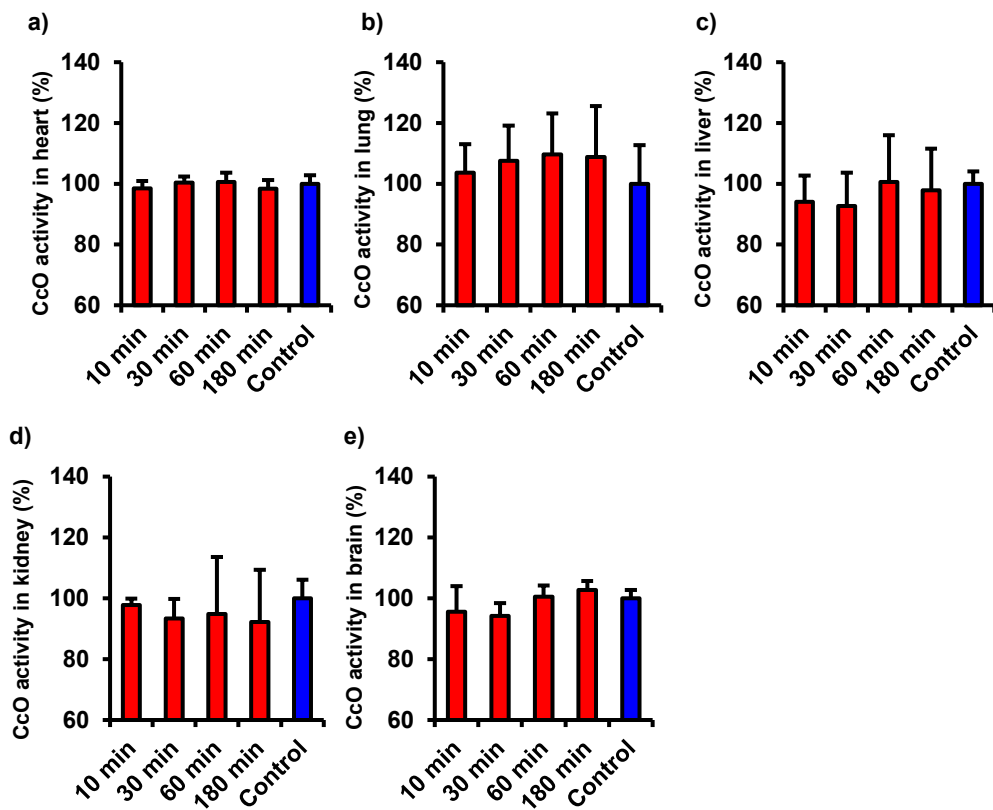
**Fig. S2. Inflammatory responses after administering metHb-albumin cluster in healthy mice.**

a) IL-1 $\beta$ , b) IL-6, c) TNF- $\alpha$ , and d) IFN- $\gamma$ . n = 4 per group, mean + S.D. S: saline



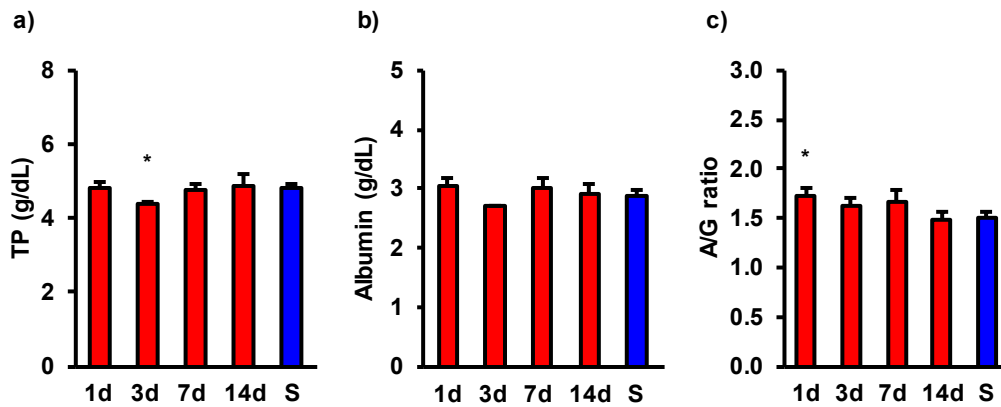
**Fig. S3. Metabolic acidosis-related blood gas parameters after an administration of H<sub>2</sub>S-metHb-albumin cluster in healthy mice.**

a) pH, b) bicarbonate (HCO<sub>3</sub><sup>-</sup>), c) lactate, and d) base excess in venous blood were determined at 10-, 30-, 60-, and 180-min after 300 mg metHb/kg H<sub>2</sub>S-metHb-albumin cluster administration to healthy mice. n = 3, mean + S.D. \*\*p < 0.01 vs. control.



**Fig. S4. Cytochrome *c* oxidase (CcO) activity after an administration of H<sub>2</sub>S–metHb-albumin cluster in healthy mice.**

Cytochrome *c* oxidase activity in **a)** heart, **b)** lung, **c)** liver, and **d)** kidney and **e)** brain were measured at 10-, 30-, 60-, and 180-min after 300 mg metHb/kg H<sub>2</sub>S–metHb-albumin cluster administration to healthy mice. n = 3, mean + S.D.



**Fig. S5. Plasma biochemistry associated with hepatic function after the administration of H<sub>2</sub>S-metHb-albumin cluster to healthy mice.**

H<sub>2</sub>S-metHb-albumin cluster (300 mg metHb/kg) was administered to healthy mice (n = 20). Four mice each were sacrificed to blood samples for evaluations of a) total protein (TP), albumin, and albumin/globulin (A/G) ratio on day 1, 3, 7, and 14. Mice received saline were sacrificed on day 14 (n = 4). \**p* < 0.05 vs. saline. S: saline