Supplementary Fig. S1 Effects of recombinant human soluble thrombomodulin (rhsTM) on brain high-mobility group box 1 (HMGB1) levels after cerebral ischaemia in mice subjected to middle cerebral artery occlusion (MCAO). Quantitative analysis of brain HMGB1 levels in the sham, vehicle and rhsTM 5 mg/kg (n = 6–8, respectively) groups. Tissue samples were collected 1, 3, 7 and 10 days after MCAO. Tissue samples of the striatum were homogenized at 4°C for 1 minute in PRO-PREP Protein Extraction Solution (iNtRON Biotechnology, Korea). The tissue extract was centrifuged at 15,000 revolutions per minute (rpm) at 4°C for 30 minutes, and the supernatant was collected. The total protein concentration of each tissue lysate was determined using Protein Assay Bradford Reagent (Wako Pure Chemical Industries, Fukuoka, Japan). Tissue protein levels were measured by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer’s instructions. *p < 0.05 versus vehicle (Student’s t-test); ††p < 0.01 versus sham. All values are means ± standard error of the mean (SEM).

Supplementary Fig. S2 High-mobility group box 1 (HMGB1) levels and survival after cerebral ischaemia. (A) Quantitative analysis of systemic HMGB1 levels after middle cerebral artery occlusion (MCAO) (n = 6–8). Blood samples were collected 1, 3, 7 and 14 days after MCAO. Plasma HMGB1 levels were measured by enzyme-linked immunosorbent assay (ELISA). (B) Quantitative analysis of survival in sham (n = 6) and vehicle (n = 16 at day 1). ††p < 0.01 versus sham. All values are means ± standard error of the mean (SEM).

Supplementary Fig. S3 Effects of recombinant human soluble thrombomodulin (rhsTM) on tumour necrosis factor (TNF)-α levels after cerebral ischaemia. Quantitative analysis of brain TNF-α levels in the sham, vehicle and rhsTM 5.0 mg/kg (n = 8) groups. Tissue samples of the striatum were collected 1, 3 and 7 days after middle cerebral artery occlusion (MCAO), and were homogenized at 4°C for 1 minute in PRO-PREP Protein Extraction Solution (iNtRON Biotechnology, Korea). The tissue extract was centrifuged at 15,000 revolutions per minute (rpm) at 4°C for 30 minutes, and the supernatant was collected. The total protein concentration of each tissue lysate was determined using Protein Assay Bradford Reagent (Wako Pure Chemical Industries, Fukuoka, Japan). Tissue protein (15 μg) was measured by enzyme-linked immunosorbent assay (ELISA) (R&D System, Inc., Minnesota, United States) according to the manufacturer’s instructions. *p < 0.05 versus vehicle (Student’s t-test); †p < 0.05, ††p < 0.01 versus sham (Tukey’s test). All values are means ± standard error of the mean (SEM).