SUPPLEMENTAL MATERIALS AND METHODS

Recombinant ADAMTS-13

The study was conducted using recombinant human ADAMTS-13, which also cleaves mouse VWF.[1,2] rADAMTS-13 was made as previously described.[3] Briefly, human *ADAMTS13* cDNA was cloned into the mammalian expression vector pSectag (Invitrogen, Carlsbad, CA) that carries the C-terminal His and Myc tags. The cDNA was introduced to Chinese Hamster Ovary cells (CHO, ATCC, Manassas, VA) by Lipofectamine TM 2000 (ThermoFisher Scientific, Carlsbad, CA). Cells stably expressing rADAMTS-13 were maintained in Dulbecco-modified Eagle medium (ThermoFisher) supplemented with 10% fetal bovine serum and 400 μg/ml of hygromycin B (ThermoFisher) until confluence. rADAMTS13 was purified from the supernatant of confluent cells in serum-free medium (Opti-Pro SFM; ThermoFisher) using a Y-per 6xHis Fusion Protein Purification Kit (Pierce Chemicals, Rockford, IL).

Measurement of plasma rADAMTS-13

Levels of rADAMTS-13 in mice receiving the metalloprotease was measured using a capturing ELISA. Briefly, 96-well nickel coated plates (Thermo scientific) were blocked with 2.5% bovine serum albumin in PBS for 1 hr at room temperature and incubated with platelet-poor plasma (PPP) collected at baseline and at 3 and 6 hrs after FPI and dilated 10-fold with PBS containing 0.5% of BSA and 0.05% of Tween-20 (PBS-BSA-Tween-20) for 2 hrs at 37℃. The incubation allowed rADAMTS-13 being captured to the

Nickel coated surface. The wells were washed with PBS-BSA-Tween-20 and then incubated with a goat antibody made against a synthetic peptide from the metalloprotease domain of ADAMTS-13 (Bethyl Laboratories) for 1 hr at room temperature. After washing with PBS-BSA-Tween-20, the plates were incubated with a HRP-conjugated anti-goat IgG (Thermo scientific) for 60 min at room temperature and, after washing with the substrate TMB for 20 min at 37℃. The HRP-TMB reaction was detected at OD 450 nm in a plate reader (Molecular Device)

Tail bleeding

Blood loss from a tail cut was measured using hemoglobin as the marker.[4] Briefly, a mouse was anesthetized by a mixture of isoflurane and oxygen (1:1) at a gas flow of 2L/ml and placed on a temperature-control surgical platform (Harvard Apparatus). Its tail was cut off at 1/10 distance from the tip using a sharp scissor. The tail end was emerged in 50 ml PBS pre-warmed to 37℃ for 20 min. The PBS was centrifuged at 4,000 rpm for 5 min at room temperature to collect erythrocytes, which were lysed with 2 ml of the red blood cell lysis buffer for 10 min. The buffer was then centrifuged at 10,000xg for 5 min at room temperature to collect the supernatant. Tail bleeding was defined as the amount of hemoglobin in the supernatant that was measured at OD 550 nm in a plate reader (Molecular Device).

We did not use thromboelastography, which has been widely used to define hemostatic defects found in patients with trauma, to analyze mouse blood samples for three reasons. First, there is no standard reference for normal values of mouse thromboelastogram so that we will be able to detect hemostatic abnormalities found in TBI mouse. In fact, there is no published report on using TEG on mice in trauma or TBI settings. Second, there is no standardized means using TEG to study the impact of cellular microvesicles on hemostasis in mice. Third, a standard TEG assay needs minimally 0.5 ml of blood (approximately 50% of mouse circulating blood), which is impossible to collect longitudinal samples from TBI mice, unless they are scarified.

SUPPLEMENTAL FIGURES AND LEGENDS

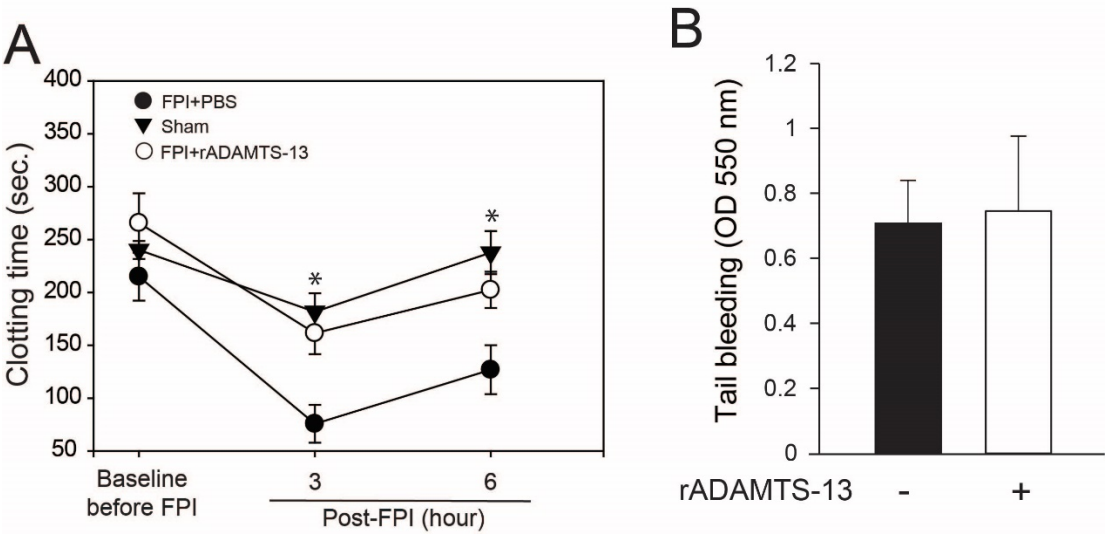

Figure S1: (A) MVs were purified from C57BL/6J mice preconditioned with either rADAMTS-13 or PBS before FPI or sham surgery, and tested for their abilities to clot phospholipid-depleted plasma in the presence of the activated coagulation factor Xa (n = 12, One-way ANOVA, *p < 0.01, PBS vs. rADAMTS-13 and sham mice).[5] **(B)** The tail bleeding of non-injured mice before and after infused with 200 μg/kg of rADAMTS-13 (n = 12, paired t test).

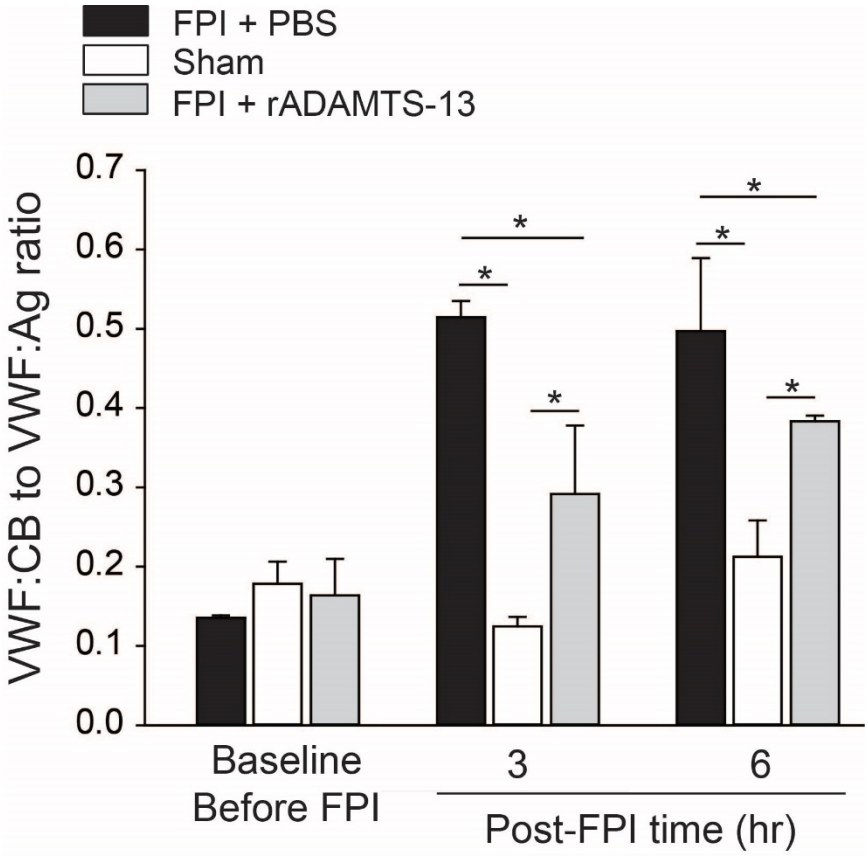

Figure S2: VWF:CB to VWF:Ag ratios of mice pre-conditioned with either PBS or rADAMTS-13 before FPI and those underwent sham surgery (n = 27, one-way ANOVA, *$p < 0.01$). This ratio defined the intrinsic VWF adhesive activity after adjustment for plasma levels of VWF antigen.

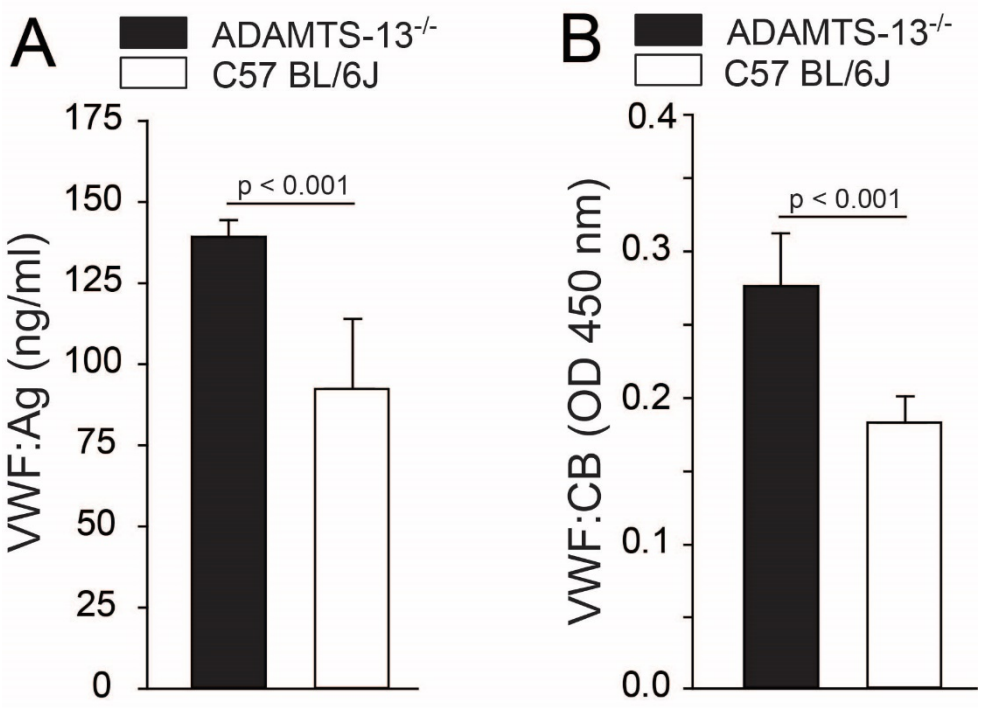

Figure S3: Baseline VWF:Ag and VWF:CB of ADAMTS-13$^{-/-}$ mice on CASA background and C57BL/6J mice without injury (n = 18, paired t test). VWF:CB was measured after adjustment for VWF:Ag with plasma from VWF$^{-/-}$ mice to detect the intrinsic VWF adhesive activity.

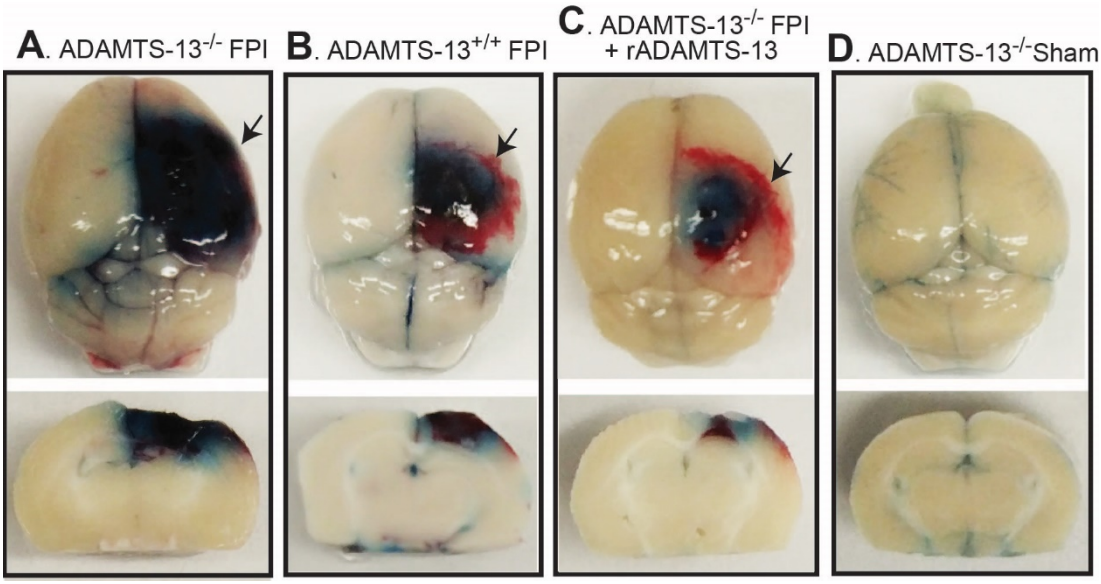

Figure S4: Evans blue dye extravasation of brains from ADAMTS-13$^{-/-}$ mice pre-conditioned with either rADAMTS-13 or an equal volume of PBS before FPI. Two controls were ADAMTS-13$^{-/-}$ mice received sham surgery or C57BL/6J mice received FPI (images are representative of 16 separate experimental mice).

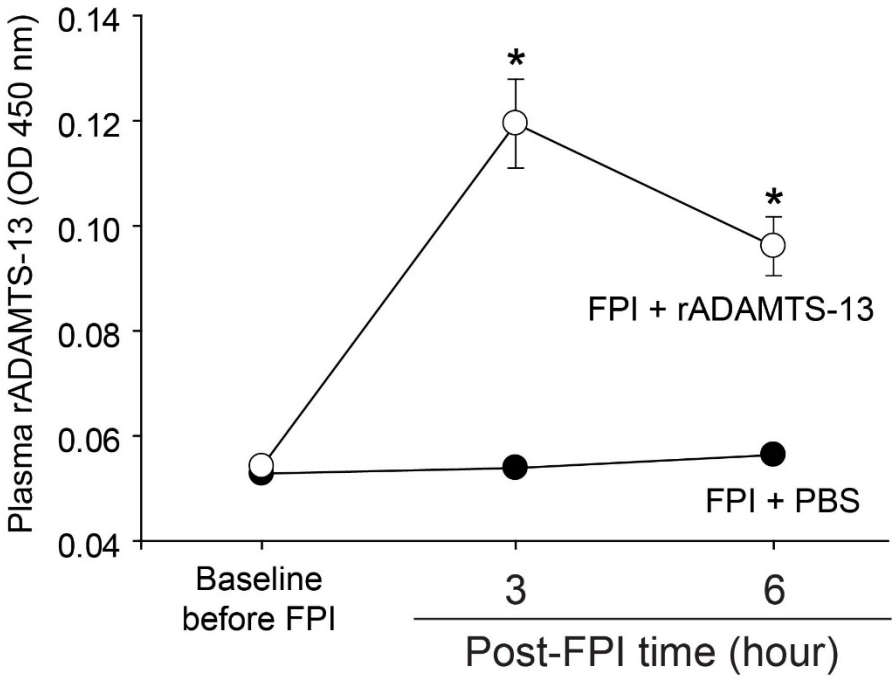

Figure S5: Plasma level of rADAMTS-13 measured in mice treated with either the metalloprotease or the vehicle control PBS (n = 6, One-way ANOVA, *$p < 0.01$).

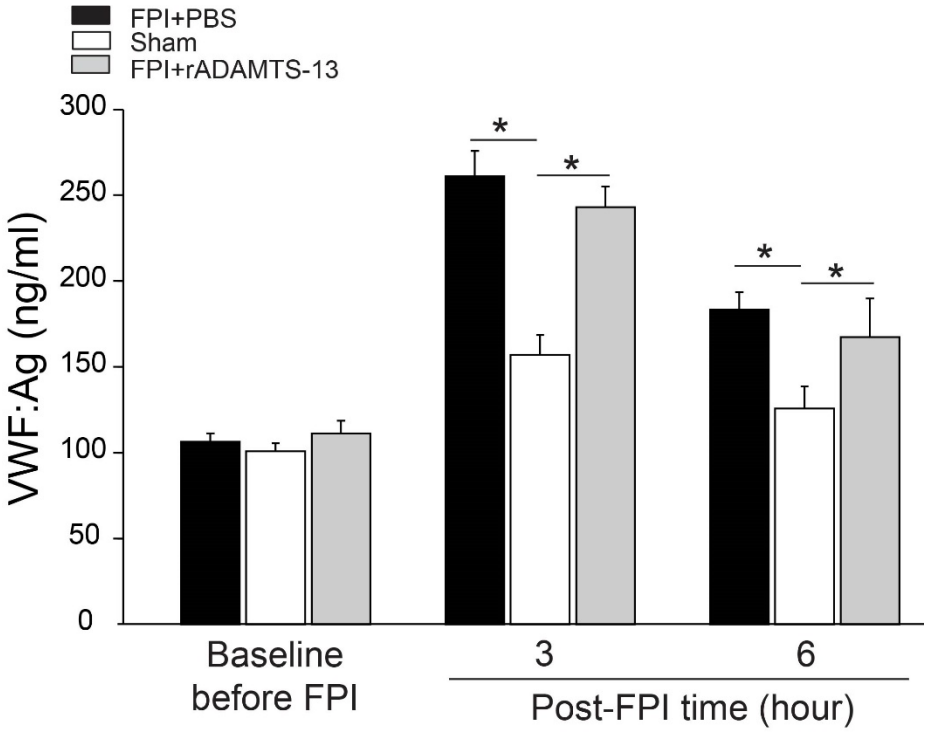

Figure S6: Plasma VWF:Ag of C57 BL/6J mice that received rADAMTS-13 or PBS after being subjected to FPI. Control mice received PBS before undergoing sham surgery (n = 18, One-way ANOVA, *$p < 0.001$).

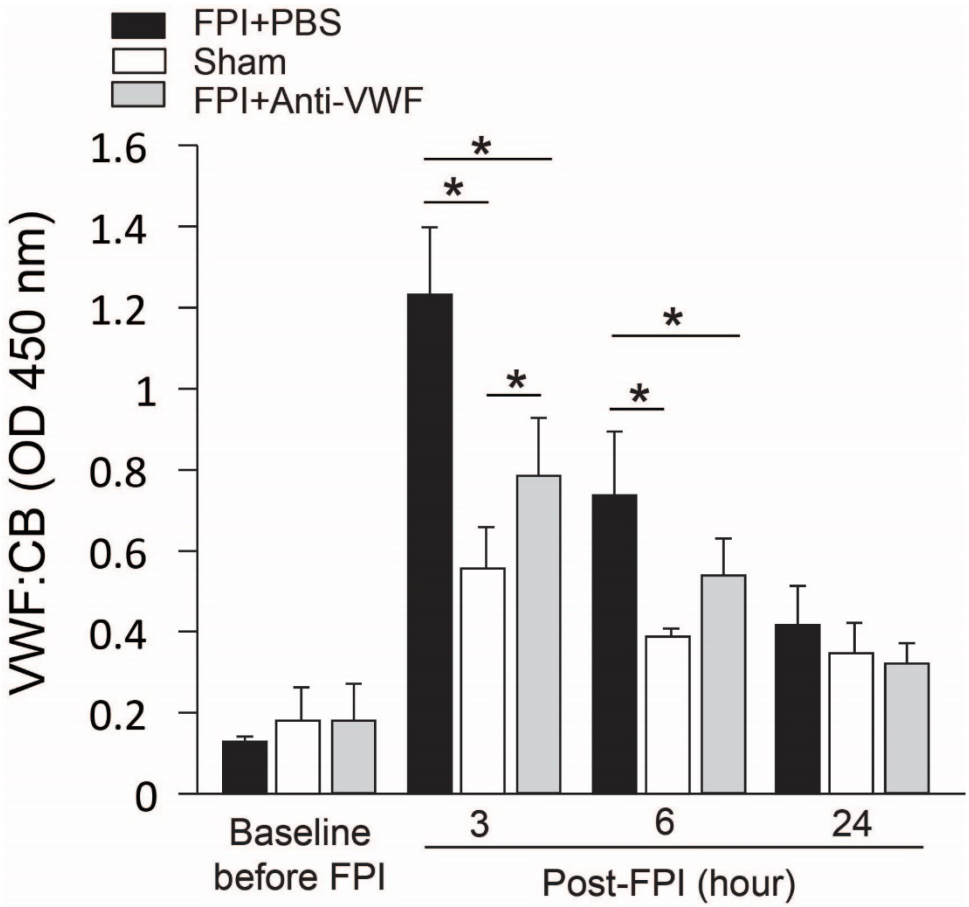

Figure S7: The FPI-induced increase in VWF:CB was reduced by the infusion of a polyclonal VWF antibody (0.78 mg/ml, 100 µl/mouse infused) 30 min after injury (n = 12, One-way ANOVA, *$p < 0.01$).

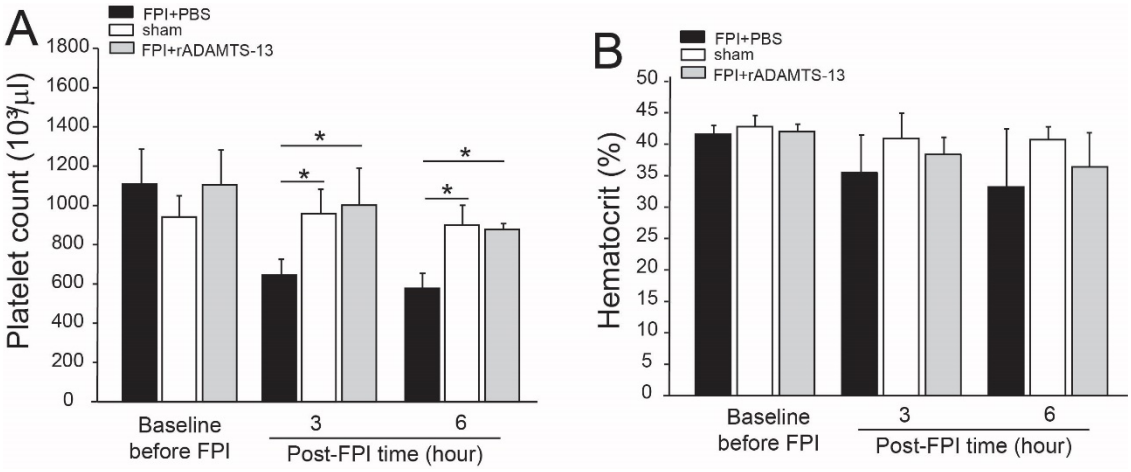

Figure S8: (A) FPI induced moderate thrombocytopenia in C57BL/6J mice that was prevented by ADAMTS-13 given 30 min after FPI (n = 12, One-way ANOVA, * $p < 0.05$) and **(B)** without causing significant changes in hematocrit (n = 12, One-way ANOVA).

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3. Tao Z, Peng Y, Nolasco L, et al. Recombinant CUB-1 domain polypeptide inhibits the cleavage of ULVWF strings by ADAMTS13 under flow conditions. *Blood*. 2005;106(13):4139-4145.
4. Liu Y, Jennings NL, Dart AM, Du XJ. Standardizing a simpler, more sensitive and accurate tail bleeding assay in mice. *World J Exp Med*. 2012;2(2):30-36.
5. Tian Y, Salsbery B, Wang M, et al. Brain-derived microparticles induce systemic coagulation in a murine model of traumatic brain injury. *Blood*. 2015;125(13):2151-2159.