Supporting Information

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SI Materials and Methods

ADAMTS13 and VWF Proteins. Recombinant ADAMTS13 (1) was obtained from Baxter Innovations. Purified human plasma VWF (Laboratoire Français du Fractionnement et des Biotechnologies, Lille, France) was chromatographed on a Superdex 200 equilibrated with 50 mM Hepes (pH 7.4), 150 mM NaCl, 5 mM $CaCl_2$, and 1 μM $ZnCl_2$ to remove traces of human serum albumin. Trace amounts of residual ADAMTS13 were removed by adsorption on monoclonal antibody 3H9-agarose.

VWF fragments SPI, SPII, and SPIII were prepared by digestion of plasma VWF with Staphylococcal V8 protease (Thermo Scientific Pierce) and purified by ion exchange chromatography (2).

Human cDNA constructs encoding VWF domain D4 (Ser^{1873}-Thr^{2255}) (3), full-length mature ADAMTS13 (4), and ADAMTS13 truncated after domain S (Ala^{685}, MDTCS), T7 (Arg^{1075}, M-T7), or T8 (Ala^{1191}, M-T8), with or without the mutation E225Q, were cloned into pTriEx-7 Ek/LIC (EMD Millipore). These constructs encode the following (underlined) mouse IgM signal peptide, StrepTag II, and enteropeptidase recognition sequence followed by Ser^{1873} of VWF or Ala^{75} of ADAMTS13:

MKFSWVMFFLMAVVTGVNSEVQASWSHPQFEKGADDDDKM

Proteins were expressed in stably transfected T-REx 293 cells (Invitrogen) grown in Freestyle serum-free medium containing 1 μg/mL tetracycline (5). Conditioned medium was adsorbed on Q-Sepharose, and proteins were eluted with 25 mM Tris·HCl (pH 8.0) and 1 M NaCl. Pooled fractions containing VWF D4 or ADAMTS13 variants were desalted on PD-10 equilibrated with 100 mM Tris·HCl (pH 8.0) and 150 mM NaCl, adsorbed on StrepTactin-agarose, and eluted with buffer containing 2.5 mM desthiobiotin.

Construct D4-CK in vector pcDNA3.1 (Invitrogen) encodes VWF residues Met^1-Cys^{22} and Gly^{1874}-Lys^{2813} followed by a 6xHis tag. Constructs A1-CK in vector pSV7D (6) and A1-CK(G1505E) in vector pcDNA3.1 encode VWF residues Met^1-Cys^{22} and Glu^{1260}-Lys^{2813} followed by a 6xHis tag. These three VWF constructs contain the mutation C2773A to prevent C-terminal dimerization (7). A1-CK(G1505E) and D4-CK were expressed in stably transfected HEK293 cells. A1-CK was expressed in transiently transfected BHK cells. Conditioned Freestyle serum-free medium was adsorbed on Q-Sepharose, and proteins were eluted with 20 mM Hepes (pH 7.4) and 1 M NaCl. Appropriate pooled fractions were dialyzed against 20 mM Hepes (pH 7.4) and 150 mM NaCl. Imidazole (20 mM) was added, and proteins were adsorbed on HisPur Cobalt agarose (Thermo Scientific Pierce). After washing with 20 mM Hepes (pH 7.4), 150 mM NaCl, and 20 mM imidazole, proteins were eluted with buffer containing 300 mM imidazole.

All proteins were further purified by chromatography on a TSK-G2000SW or Superdex 200 and stored at −80 °C. The concentration of proteins was determined in BCA protein assays (Thermo Scientific Pierce) standardized with BSA.

For epitope localization, ADAMTS13 constructs were cloned in pcDNA4/TO with C-terminal V5 and $(His)_6$ tags and expressed transiently in T-REx 293 cells (8). C-terminal deletions were truncated after domain M (Gln^{289}), D (Gly^{385}), T1 (Glu^{439}), C (Cys^{555}), S (Ala^{685}), T2 (Tyr^{745}), T3 (Arg^{807}), T7 (Arg^{1075}), T8 (Ala^{1191}), or CUB1 (Ala^{1291}). Internal deletions lacked domain T2 (Trp^{686}-Tyr^{745}), T3 (Trp^{746}-Arg^{807}), T4 (Trp^{808}-Ala^{894}), T5 (His^{895}-Pro^{952}), T6 (Ala^{953}-Arg^{1015}), T7 (Trp^{1016}-Arg^{1075}), T8 (Trp^{1076}-Ala^{1191}), or CUB1 (Cys^{1192}-Glu^{1298}).

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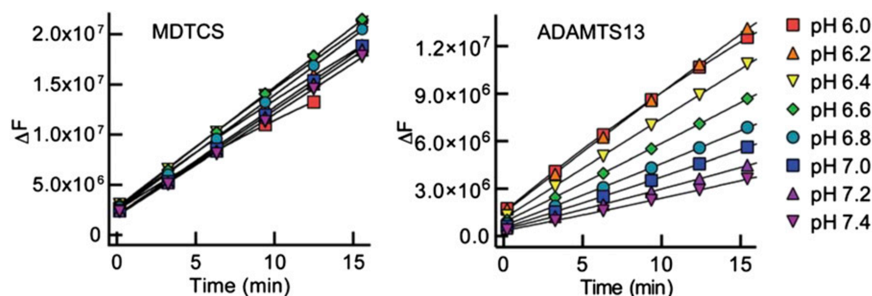

Fig. S1. Reaction time course for MDTCS and ADAMTS13 as a function of pH. Enzymes MDTCS and ADAMTS13 were assayed with substrate VWF71 at the indicated pH values.

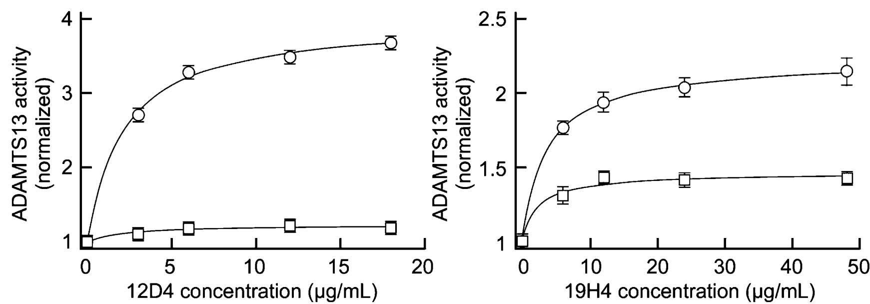

Fig. S2. Dependence of ADAMTS13 activation on monoclonal antibody concentration and pH. Concentration dependence of ADAMTS13 activation at pH 7.4 (open circles) by monoclonal antibody 12D4 (*Left*) or 19H4 (*Right*). At pH 6.0 (open squares), ADAMTS13 activity was less affected by either antibody. Antibody 12D4 recognizes ADAMTS13 CUB domains, and antibody 19H4 recognizes ADAMTS13 T8 domain. ADAMTS13 activity values are expressed as the ratio of the VWF71 cleavage rate in the presence/absence of antibody. Error bars indicate 95% confidence intervals.

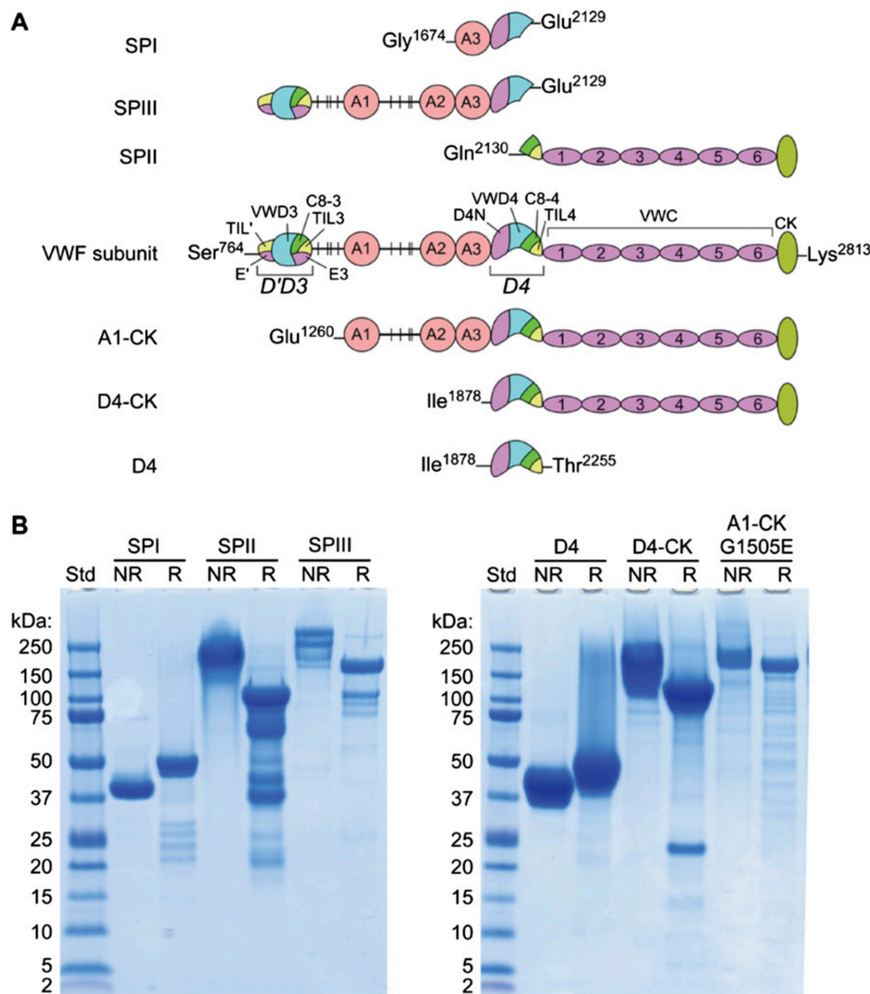

Fig. S3. Structure of VWF, V8 protease digestion products, and recombinant VWF fragments. (*A*) Dimensions of the VWF subunit are to scale based on EM data, and the total length is ~65 nm (1). Reannotated D3 and D4 assemblies contain VWD (blue), C8 (green), TIL (yellow), and E or D4N domains (violet); D′ consists of TIL and E domains. Other domains are VWA (pink), VWC (violet), and CK (chartreuse). E, D4N, and C domains are homologous. Whiskered lines are O-glycosylated segments. Staphylococcal V8 protease cleaves after Glu^{2129} (2) at the boundary between domains VWD4 and C8-4 to generate fragments SPII and SPIII as indicated. Additional cleavage after Glu^{1673} between domains A2 and A3 produces fragment SPI. Recombinant A1-CK and D4-CK have the CK domain mutation C2773A and are monomeric. (*B*) Gel electrophoresis of purified VWF proteolytic fragments and recombinant VWF proteins.

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Fig. S4. Binding of MDTCS and ADAMTS13 to VWF constructs. BLI sensorgrams, steady-state analyses, and K_d values ± SE are shown for MDTCS(E225Q) (*Left*) and full-length ADAMTS13(E225Q) (*Right*) binding at pH 7.4 to the indicated VWF derivatives.