SUPPLEMENT

FIGURE LEGENDS

Figure S1: VWF as a cofactor for fI-mediated C3b cleavage. C3b was incubated with fI and (A) increasing concentrations of plasma-purified VWF (1-20 µg/ml) for 60 min or (B) with 1 µg/ml of VWF for different incubation intervals (5-720 min). VWF concentration close to its normal plasma concentration (10 µg/ml) showed maximum cofactor activity, and half of C3b was cleaved after 10-30 min incubation with fI and 1 µg/ml VWF.

Figure S2: The effect of glycocalcin, factor VIII, ADAMTS-13, or anti-VWF antibody on the cofactor activity of VWF. C3b (1 µg) was incubated with factor I (0.5 µg) and plasma-purified VWF (1 µg) in the presence of different concentrations of glycocalcin, factor VIII, ADAMTS-13, or anti-VWF antibody. Cleavage products of C3b were separated by SDS-PAGE and Western-blotted using anti-C3 antibody. The density of bands representing intact α’ chain was measured with ImageJ analysis software (http://imagej.nih.gov/ij). The results were normalized to that of C3b cleavage in the presence of plasma-purified VWF and fI (designated as 100% cleavage). Addition of anti-VWF antibody, and to a lesser degree ADAMTS-13, reduced C3b cleavage. Anti-VWF antibody (4 µg/ml) and ADAMTS-13 (4 µg/ml) reduced fI/VWF-mediated C3b cleavage by 87±6% and 54±3%, respectively (n=3, t-test, p<0.05).

Figure S3: C3b bind to VWF. (A) Co-immunoprecipitation assay: Plasma-purified VWF (pVWF) (5 µg) was incubated with purified C3b (5 µg), immunoprecipitated with anti-VWF antibody or control IgG, and immunoblotted with anti-C3 (upper panel) or anti-VWF (lower panel). (B) Surface plasmon resonance (SPR) assay: VWF-coated sensor surface (about 1700 RU) was prepared by injection of 40 µg/ml of plasma-purified VWF (in 100 mM NaOAc, pH5.5) on a CM5 chip using a standard immobilization protocol for Biacore 3000 system. A reference surface was prepared without any protein immobilized. SPR signals from the binding of different concentrations of soluble C3b (dialyzed in running buffer composed of 10 mM Tris, pH7.0, 125 mM NaCl, and 5 µM ZnCl₂) to the immobilized VWF were measured. (C) The equilibrium dissociation constant (K_D) was calculated based on the binding isotherm.

Figure S4: Hydrodynamic shear forces promote binding of C3b to VWF. Purified C3b (5 µg) and VWF (5 µg) were mixed in PBS buffer and exposed to shear force of 0 or 120 dyes for 2 min, and immunoprecipitated using anti-VWF antibody or IgG. The co-immunoprecipitated proteins were separated by SDS-PAGE, and Western-blotted using anti-C3 or anti-VWF antibodies. The data was analyzed by ImageJ analysis software, which revealed 2-fold increase in the relative density of bands representing C3b co-immunoprecipitated with VWF (results were normalized to the density of VWF bands). A sheared sample immunoprecipitated with IgG was shown as the negative control (n=2).
FIGURES

Figure S1

A

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Blot: C3

B

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Coomassie Blue staining

α'

β

68kDa

iC3b
Figure S2
Figure S3
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Blot: C3

Blot: VWF

Figure S4