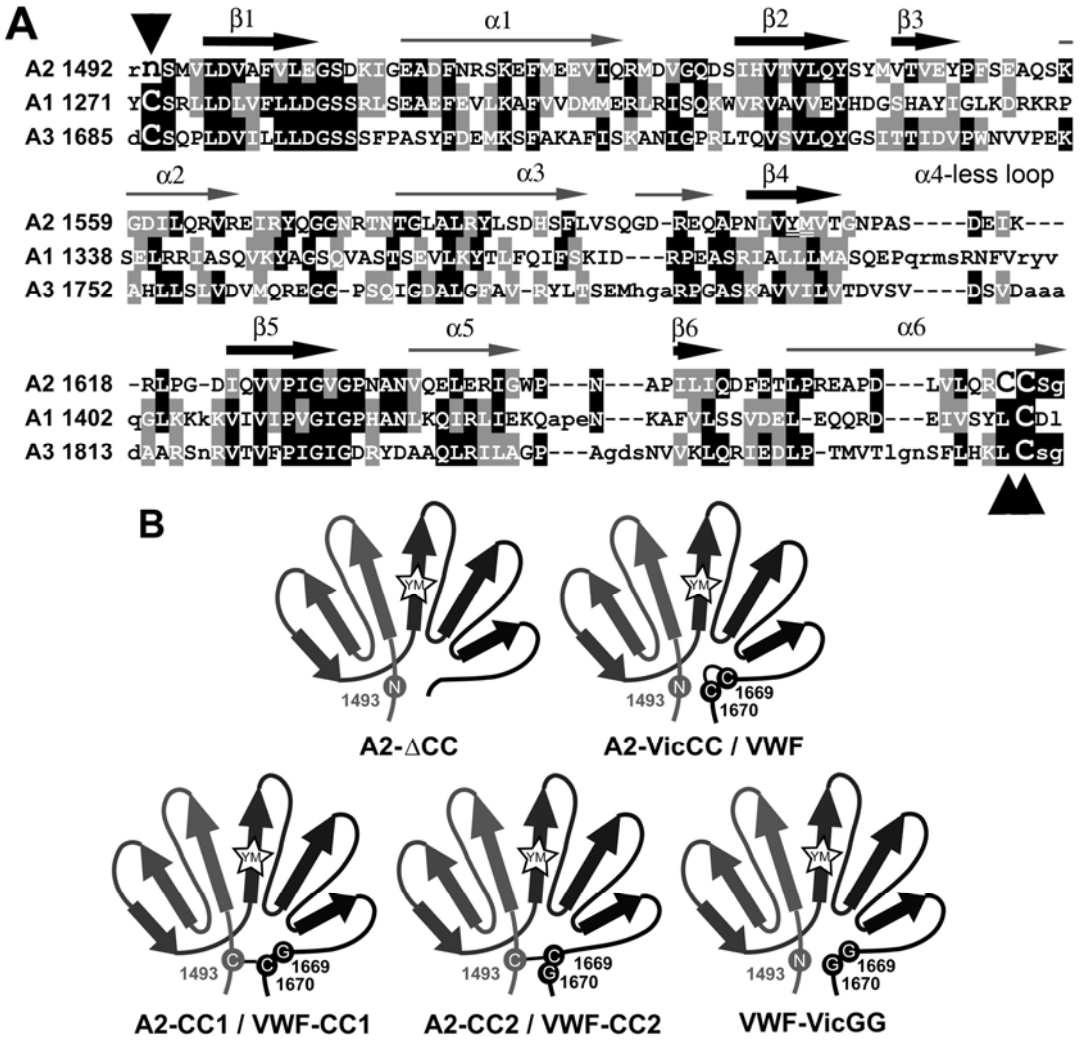

Figure S1. Position of vicinal cysteines C1669 and C1670 and the engineered disulfide bonds in the VWF A2 domain

(A) Structural alignment of the VWF A2, A1 and A3 domain amino acid sequences using pdb files 3gxb, 1auq and 1ao3, respectively and the DALI server (http://ekhidna.biocenter.helsinki.fi/dali_server). Identical and similar residues are indicated in black and grey, and were coloured using BOXSHADE. Structurally equivalent positions are given in uppercase, insertions and residues at the N/C-terminus that were not included in the structures are in lowercase. The vicinal cysteines C1669 and C1670 at the C-terminus of the VWF A2 domain are shown in large font, as are the cysteines at the N and C-terminus of the VWF A1 and A3 domains that form an intradomain disulfide bond. Residue N1493 of the VWF A2 aligns with the positions of the N-terminal cysteines in VWF A1/A3 and was changed to cysteine to introduce an engineered disulfide bond in VWF A2, this residue is also indicated in large font. α-helices and β-sheets are denoted by arrows. (B) Graphical representation of VWF A2 and variants thereof, as used as domain fragment or part of full-length VWF in this study. The β-strands that form the central β-sheet are indicated by arrows, the buried Y1605-M-1606 cleavage site is indicated as star, the residues involved in disulfide bond formation in each VWF A2 construct are indicated by closed circles at the bottom of the VWF A2 domain.

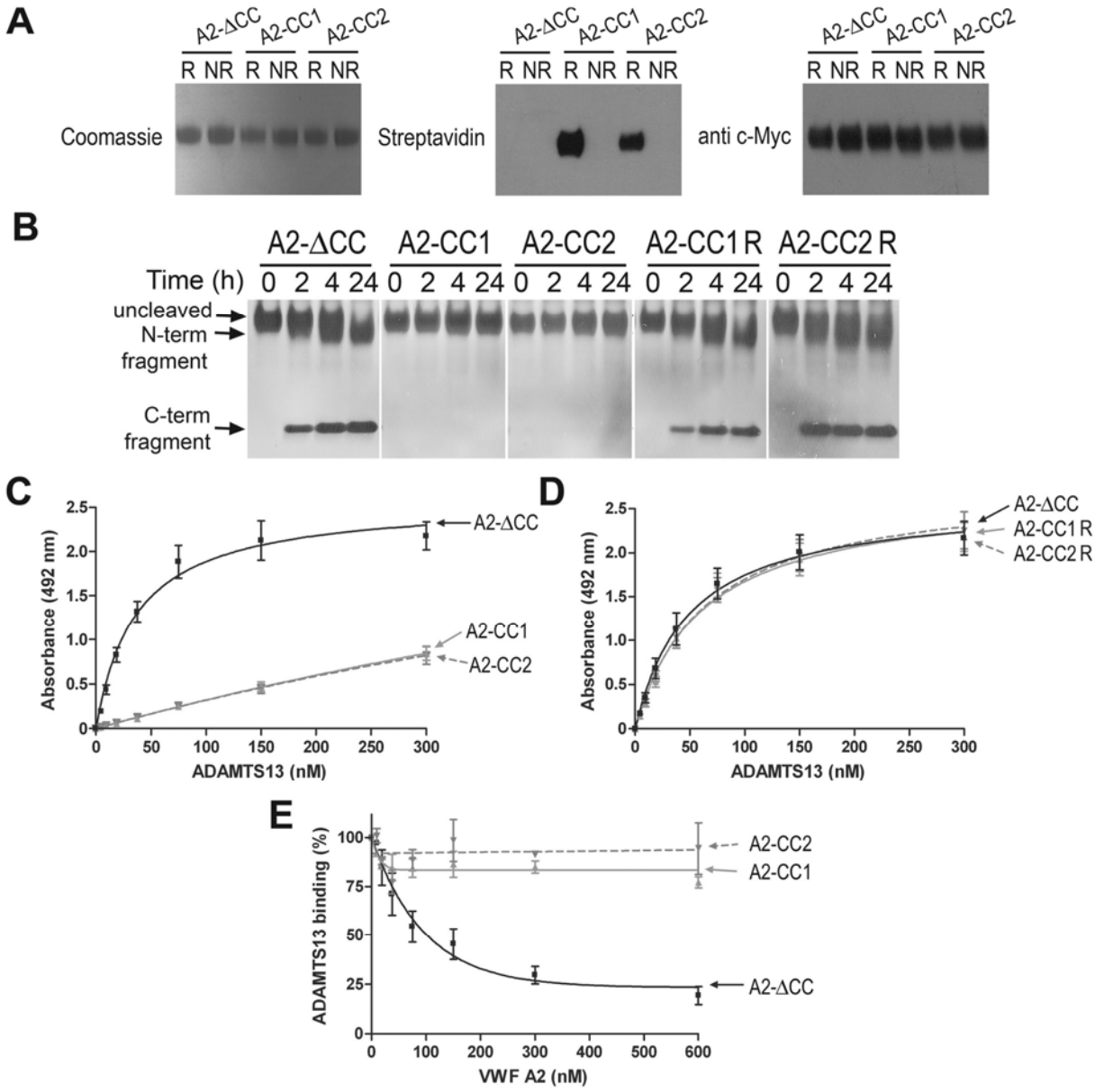

Figure S2. Analysis of VWF A2-ΔCC, A2-CC1, and A2-CC2 with engineered disulfide bonds (A) Left panel: Coomassie stained SDS-PAGE gel with the purified VWF A2 protein fragments, non-reduced (NR) and reduced (R) prior to analysis. Middle panel: VWF A2 fragments were incubated with biotin-labeled maleimide with/without prior reduction. Biotin-labeled sulfhydryl groups were detected on Western blot using peroxidise-labeled streptavidin and were only present on VWF A2-CC1 and A2-CC2 after reduction. Right panel: the same samples as in B were also detected using a monoclonal antibody directed against the c-myc tag to control for equal loading. (B) VWF A2 fragments (NR) and reduced and carboxymethylated forms (R) were incubated at 37°C with 20 nM ADAMTS13 in 1.5M urea for 0–24 hours at which reactions were stopped with EDTA. Samples were reduced and separated on SDS-PAGE and visualized by silver staining. (C) Binding of ADAMTS13 to immobilized VWF A2 fragments. (D) As in (C) but VWF A2 fragments were reduced and carboxymethylated prior to use in the assay. (E) Binding of ADAMTS13 to immobilized VWF A2-ΔCC in the presence of increasing concentrations of soluble VWF A2-ΔCC, A2-CC1, and A2-CC2.