SUPPLEMENTAL MATERIAL

Supplemental Methods:

Immunoprecipitation- As we described but with some modifications [22]. As part of another ongoing project, lysate from human umbilical vein endothelial cells in Radio-Immuniprecipitation Assay (RIPA) buffer (Sigma) was mixed with purified A2 domain (300 μg/ml) or buffer and incubated for 2 hours at 37°C. Then the two lysate mixtures were incubated with 10 ug/ml anti-A2 antibody from R&D System, Inc. (Minneapolis, MN) overnight at 4°C. The Pansorbin (beads) was blocked with 10% BSA in TBS for 1h at 4°C. After washing the Pansorbin thoroughly with buffer, pH-7.4, it was incubated with goat anti-mouse antibody (Pierce) overnight at 4°C. After the incubation, the Pansorbin was washed by centrifugation with buffer and added to each lysate mixture and incubated for immunoprecipitation for 2-3 hours at 4°C. The Pansorbin was pelleted by centrifugation and the bound protein eluted by boiling in 2x sample buffer containing 2% β-mercaptoethanol. Immunoprecipitated proteins were resolved by 7% SDS-PAGE under reducing conditions and colloidal blue stained. Bands were excised from the gel and subjected to MS/MS analysis.

Binding assays–The microtiter wells were coated with vimentin (5 μg/ml) as described in the methods section. Increasing concentrations (0 – 1.0 μM) of purified A2 domain protein were added to the wells. The A2 domain bound to vimentin was detected by ELISA using the monoclonal anti-human A2 domain antibody, VP-1, followed by a secondary anti-mouse antibody horseradish peroxidase conjugate. To test the capacity of the antibody V9 in blocking the binding of A2 domain to vimentin, A2 domain (0.05 μM) was mixed with mouse IgG or V9 (0.5 μM) and incubated in microtiter wells coated with vimentin as described above. The A2 domain bound to vimentin was detected as described in previous section. In another binding assay, the microtiter wells were coated with the A1 or A2 domain as we previously described [27]. After blocking with BSA, 75 μl of the pooled fractions containing vimentin was added to each well and incubated for 1 h at 37°C. Bound vimentin was detected as described in figure 1.

Flow cytometry- To confirm that vimentin is exposed on the surface upon platelet activation we followed the protocol from the original report [25]. Citrated whole blood without or with ADP (20 μM) was incubated with either rabbit monoclonal anti-vimentin antibody (Epitomics) or rabbit IgG (Pierce) for 10 min. Then, goat anti-rabbit FITC antibody was added to all the samples and after 10 min, the samples were fixed with 1% paraformaldehyde. The samples were subjected to analysis by using immunofluorescence flow cytometry.

Platelet Adhesion under Flow- To determine whether vimentin is exposed at the surface of platelets during perfusion we performed a previously described approach [29]. The rabbit anti-human vimentin antibody or rabbit IgG and purified recombinant A1 domain were mixed and immobilized onto glass coverslips using coating solution concentrations of 200 μg/ml and 75 μg/ml, respectively. The coverslips were incubated overnight at 4°C. The coverslips were then washed to remove the unbound protein, and then blocked with 3% BSA in TBS for 1 hr at room temperature before use. The perfusion of whole blood and the determination of the translocation velocity of platelets were performed as described in methods.

Partial isolation of plasma vimentin- Human plasma from healthy donor was passed through a size exclusion column (Sepharose CL-4B) equilibrated with TBS. As expected [23], vimentin eluted from the column as a dimer (~120 kDa) and the fractions containing vimentin (determined by western blot, Fig. S4C) were pooled.

Supplemental Figures

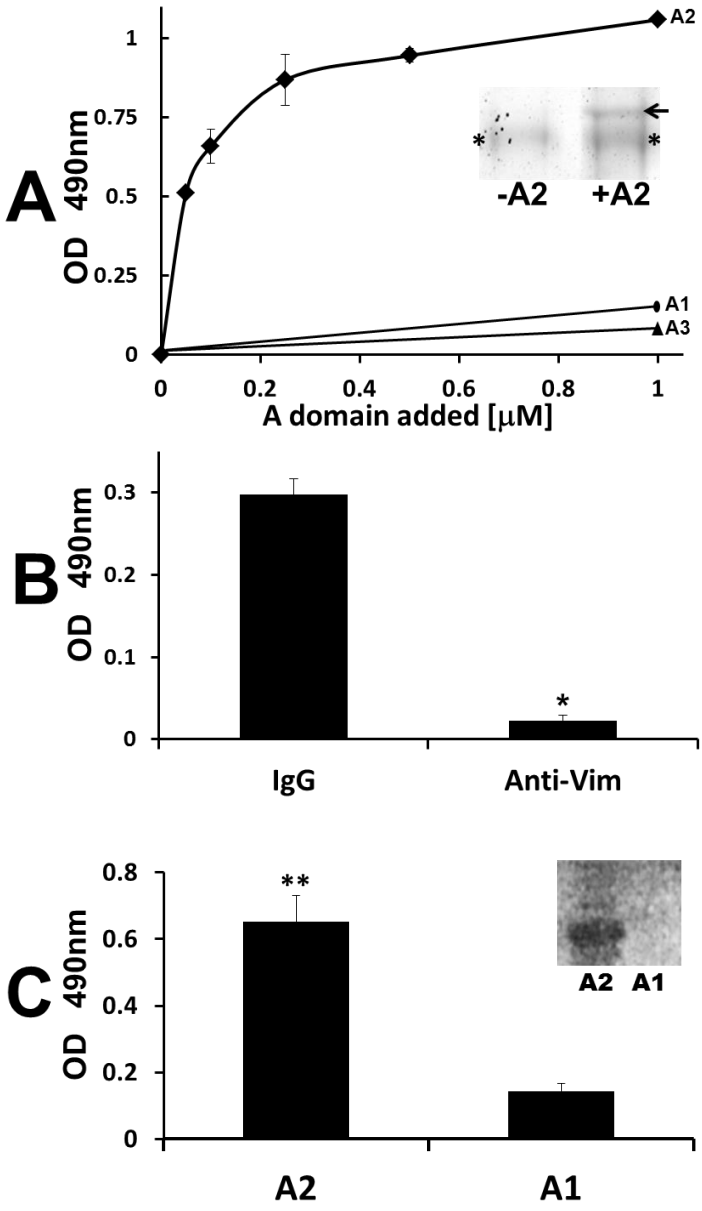

Figure S1

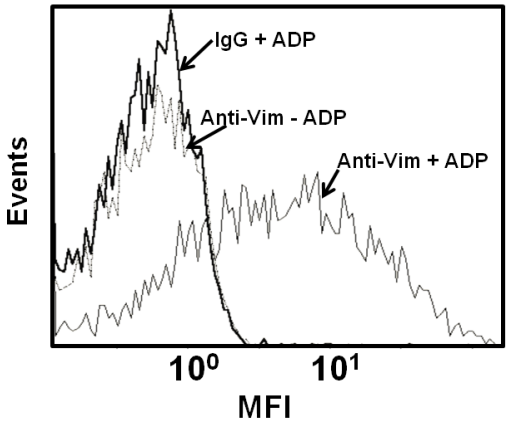

Figure S2

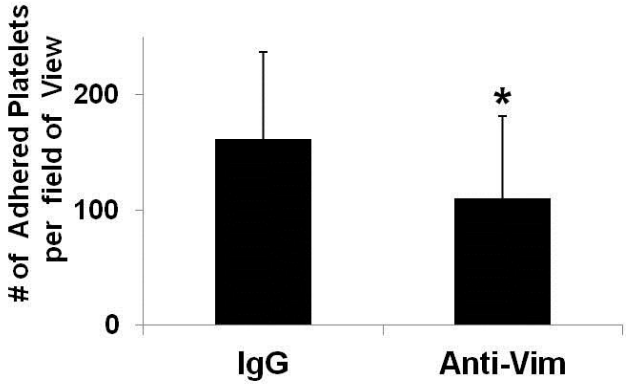

Figure S3

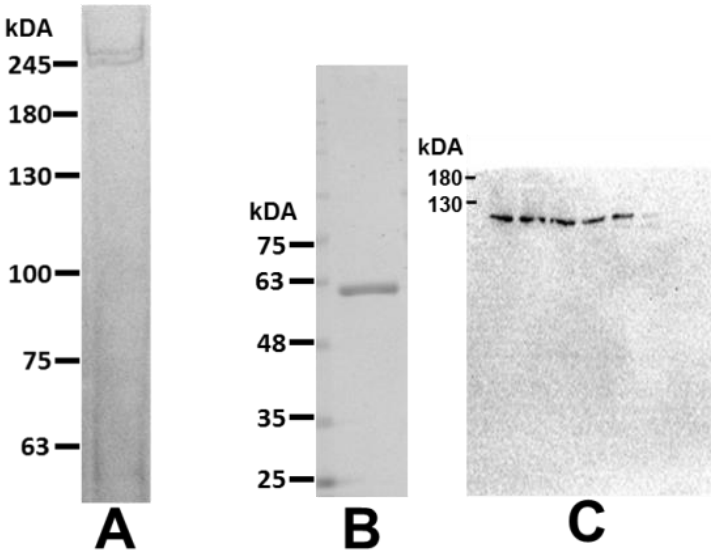

Figure S4

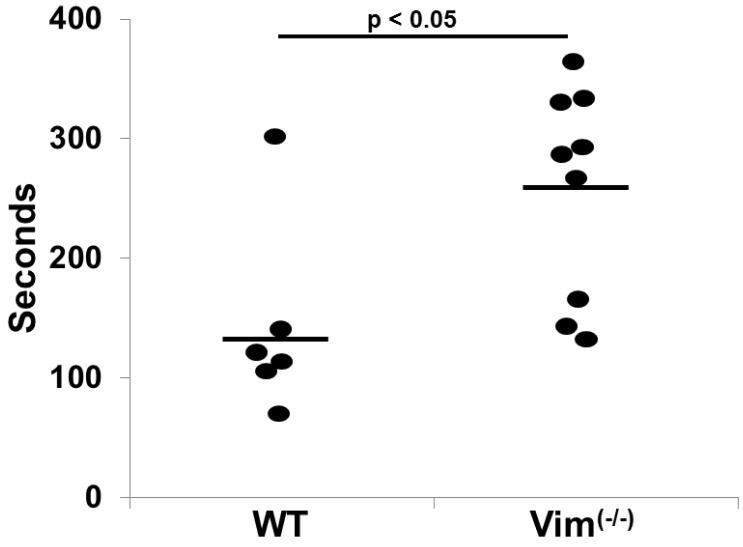

Figure S5

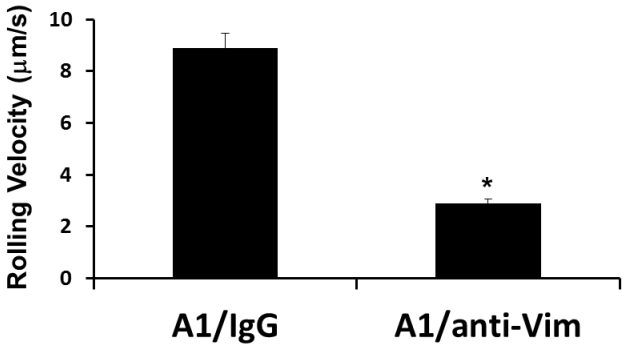

Figure S6

Figure Legends

Figure S1. (A, *inset)*- Human umbilical vein endothelial cells lysate was used in an Anti-A2 antibody pull-down assay to identify a potential A2 domain interacting proteins. Lysate was incubated with Anti-A2 antibody coated beads in the presence or absence of soluble A2 protein. Bound proteins were eluted using reduced sample buffer and analyzed by SDS-PAGE and colloidal blue stained. Protein bands were cut off from gel, trypsinized and analyzed by MS/MS. The protein band with *black arrow* was identified as vimentin. Asterisks (*) represent a band present in both lanes, which was identified as an IgG fragment. Representative of two separate experiments. **(A)** Specific binding of the A2 domain to vimentin. Increasing concentrations of the A2 domain were incubated with immobilized vimentin (5 μg/ml). The bound protein was determined by ELISA, using monoclonal antibody against human A2 domain of VWF, VP-1. The graph is representative of three separate experiments. Each A1 and A3 protein had a poor binding for vimentin at 1.0 μM. Each point represents the mean ± S.D. of values obtained from a triplicate assay, p<0.01. **(B)** Monoclonal anti-human vimentin antibody, V9 (0.5 μM), effectively blocked the binding of A2 domain (0.05 μM) to immobilized vimentin. Data represents mean ±

SD of two triplicate assays, *p<0.05. **(C,** ***inset*****)** Analyzing the vimentin-A1 or A2 domain interaction via blot overlay. Non-reduced A1 and A2 domain were transferred to the membrane followed by blocking with 5% milk in TBS. Then, the membrane was incubated with vimentin (5 μg/ml) for 1 hour and washed three times with TBS-T. The bound vimentin was detected using anti-human vimentin-HRP antibody. **(C)** Isolated vimentin from human plasma was added to wells coated with A1 or A2 domain (0.5 μg/ml) and incubated for one hour. The bound vimentin was determined by ELISA using anti-human vimentin-HRP antibody as described in figure 1. The graph shows the specific binding and the data represents mean ± SD of a quadruplicated assay, **p<0.01.

Figure S2. Vimentin was detected on the surface of activated platelets by flow cytometry as originally described by others [25]. Whole blood was incubated with either monoclonal rabbit anti-human vimentin antibody or isotype IgG in the presence or absence of ADP (20μM).

Figure S3. Whole blood was mixed with sheep IgG molecule or sheep anti-human vimentin and perfused over surfaces coated with soluble collagen Type III at shear rate of 1,500s^{-1}. After 2-min perfusion, the plates were washed with buffer and attached platelets were recorded and quantified as described in the methods. The bar graph shows the average number of the adhered platelets per field of view (10 fields) (mean ± SD, *p<0.05).

Figure S4. SDS-PAGE analyses under reduced conditions for ***A***- silverstained gel of the purified murine VWF, and ***B-*** Coomasie blue stained gel of the recombinant human vimentin. ***C-*** Western blot to detect vimentin under non-reduced conditions among the fractions eluted from the size exclusion column chromatography.

Figure S5. The lack of vimentin affects tail bleeding time. The average bleeding time for the vimentin knock out mice ($n=9$) was two fold of that of WT mice ($n=6$).

Figure S6. Perfusion of whole blood and the determination of the translocation velocity of platelets were performed as described in methods. The bar graph shows the average velocity of platelets from two separated experiments (mean ± SD, *p<0.005).