ADAMTS9 is a cell-autonomously acting, anti-angiogenic metalloprotease expressed by microvascular endothelial cells

SUPPLEMENTAL DATA FIGURES:

Supplemental Figure 1. b-gal stained cells observed in the embryonic and adult microvasculature are not vascular smooth muscle cells. A, **B**. NG2 staining of E12.5 sections showing that NG2+ cells (arrows) lack a b-gal stained nucleus and lie external to b-gal stained cells. **C**. SMA staining of adult spleen shows that the pericyte (red, arrow) encircles the b-gal stained cell (blue). **D**. SMA staining of a 5-day old excisional wound bed (brown) shows that the majority of b-gal stained cells do not express SMA. This image should be viewed alongside Fig. 2L, taken from the same wound bed, showing that the majority of b-gal stained cells are endomucin+.

Supplemental Figure 2. Effect of *ADAMTS9* siRNA on formation of tube-like structures on Matrigel by human heart microvascular EC (HHMEC) (A), human dermal microvascular EC (HDMEC) (B) and human heart umbilical vein EC (HUVEC) (C). In each sub-figure, the top two panels illustrate representative fields photographed under a phase contrast microscope showing the morphology of tube-like structures obtained using a control siRNA (left) and *ADAMTS9* siRNA (right). The bottom left-hand panel is a graphic summary of quantitative analysis of the length of tubular structures formed (showing the mean ± SD of three independent replicates). The bottom right-hand panel shows an agarose gel indicating the level of suppression of *ADAMTS9* siRNA relative to the control, and *GAPD* RNA as a standard, as indicated.

Supplemental Figure 3: Migration of A549 cells in enhanced by *ADAMTS9* siRNA. A. RT-PCR data show expression of *ADAMTS9* mRNA in A549 cells and successful knockdown with siRNA. **B**. Time-lapse images of scratch wound assay using *ADAMTS9* siRNA treated and control siRNA treated A549 cells at indicated time after wounding. **C**. Quantitative analysis of closing of the scratch is shown as a percent of 0 hr (mean ± SD of three independent replicates).

Supplemental Figure 4: *ADAMTS9* siRNA does not affect HBMEC proliferation. The data show the mean ± SD of cell proliferation measured using the WST-1 assay and compares cells transfected with *ADAMTS9* siRNA or control siRNA.

Supplemental Figure 5: Effect of over-expression of ADAMTS9 N-L2 on formation of tube-like structures on Matrigel by HUVEC. A. HUVEC were transfected with control vector (pcDNA3.1myc-His) or with ADAMTS9 N-L2 or ADAMTS9 N-L2 $Glu^{435}Ala$ ($E^{435}A$) as indicated and allowed to form tube-like structures on Matrigel. The histogram representing the mean ± SD for three independent replicates shows that although expression of ADAMTS9N-L2 and ADAMTS9 N-L2 $Glu^{435}Ala$ decrease and increase tube formation respectively, neither effect reaches statistical significance. **B**. Western blot analysis of lysates of transfected HUVEC using anti-myc to illustrate that they express the transfected plasmid. The arrow indicates the ADAMTS9 species.