Supplementary information

Supplementary Materials & Methods

Generation of α4 integrin $S^{988}A$ knock-in mice: A genomic fragment encompassing exons 25-28 of mouse α4 integrin was cloned into Bluescript plasmid. A single Ser-to-Ala point mutation was created at position 988, and a *neo* cassette flanked by loxP sequences was introduced between exon 27 and exon 28 for drug selection. The targeting construct was electroporated into embryonic stem cells and drug-resistant clones were screened by Southern blotting for recombination between the targeting construct and the α4 genomic locus. Positive clones were injected into blastocysts, which were then implanted into pseudo-pregnant female mice. Chimeric mice (agouti) were crossed with mice of the same genetic background as the blastocysts. Progeny from these crosses were screened by genomic PCR for heterozygous $S^{988}A$ mutation. Heterozygous $S^{988}A$ progeny were crossed to obtain homozygous littermates.

Generation of α4 fl/fl (null) pulmonary endothelial cells: α4 fl/fl cells were treated with soluble Cre recombinase to remove exon 28 and the polyadenylation tail from the α4 gene locus. Elimination of α4 protein expression was confirmed by FACScan using R1/2 antibodies to mouse α4 integrins (Abcam, Cambridge, MA).

Supplementary Figures

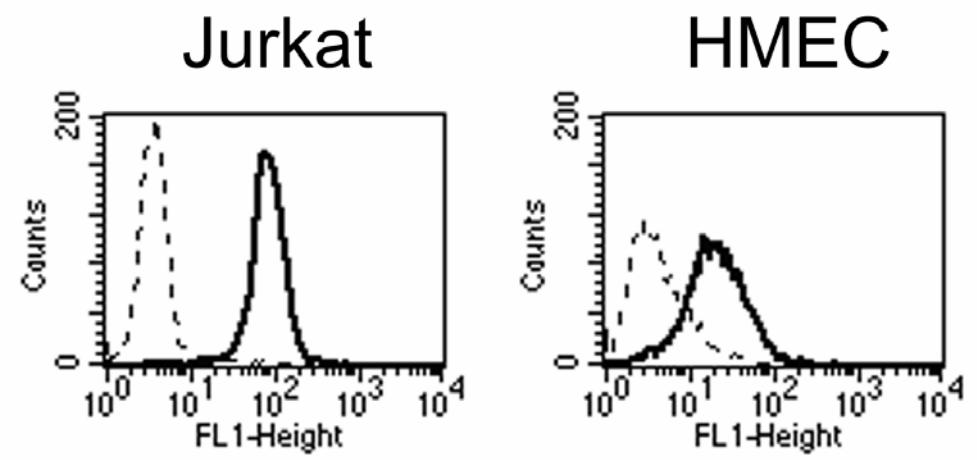
Supplementary Figure 1: Jurkat cells and human microvascular endothelial (HME) cells were surface-labeled with mouse IgG (dotted lines) or HP2/1 mouse anti-human α4 integrin and subjected to FACS analysis.

Supplementary Figure 2: HMECs were pre-incubated for 15 min with blocking antibodies against the indicated integrins and seeded onto surfaces coated with the cell-binding fragment of fibronectin (911), the α4-binding fragment of fibronectin (CS-1), collagen, or poly-L-Lysine for 60 min. Cells were fixed and labeled with crystal violet to indicate relative numbers of adherent cells.

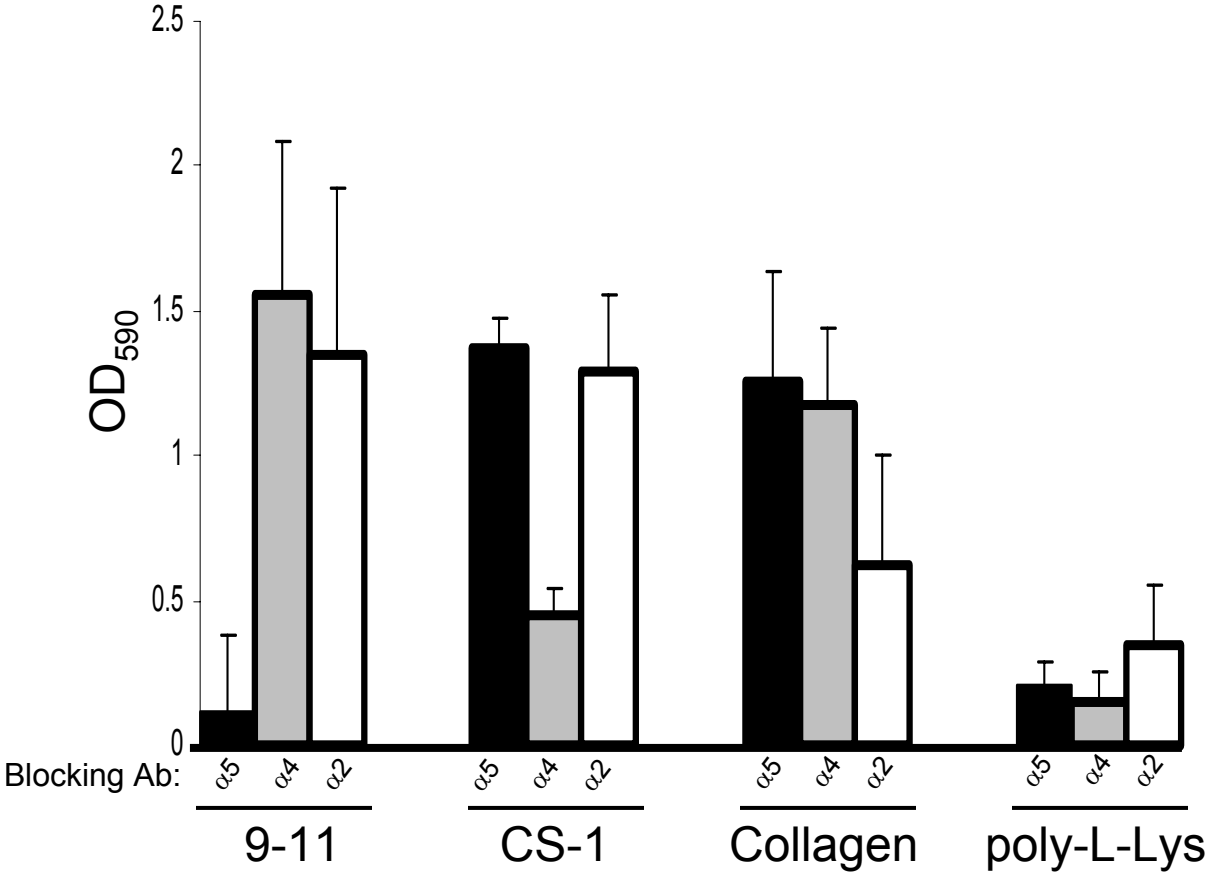
Supplementary Figure 3: Cells in Figure 5B were suspended with trypsin and labeled with R1/2 antibodies to mouse α4 integrin and analyzed by FACS.

Supplementary Figure 4: Primary endothelial cell cultures derived from mice expressing wild type α4 or α4 harboring a Ser→Ala mutation at position 988 were seeded on CS-1, scratch wounded and subjected to shear stress for 5 min. Sheared cells were fixed and stained with PSα4. White arrows indicate enhanced plasma membrane α4 phosphorylation.

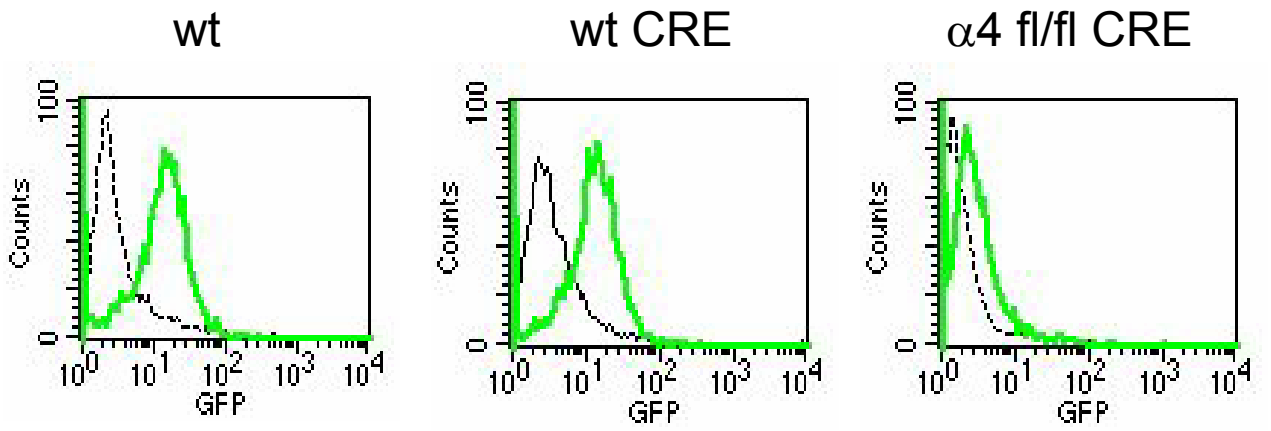
Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4