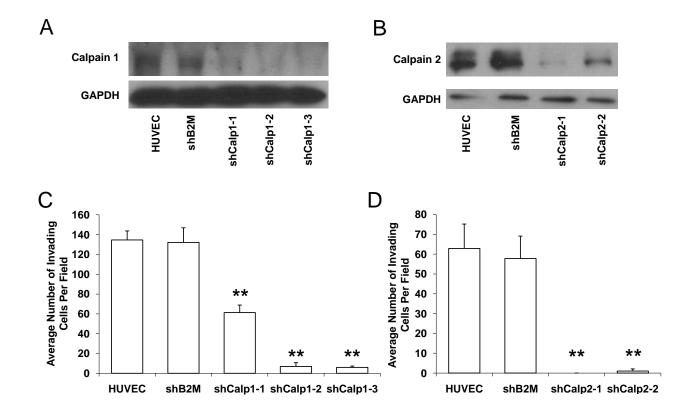


Control

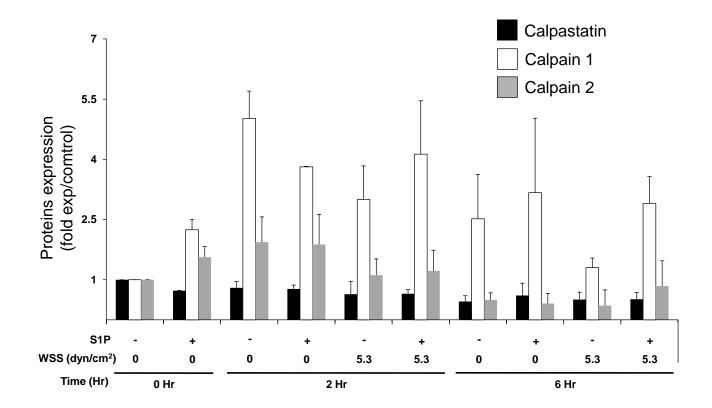
Global Calpain Inhibitor (ALLN)

Calpain inhibitor III (CI-III)

SUPPLEMENTAL FIGURE 1. ECs were seeded on collagen matrices containing 1 μ M S1P and treated with 5.3 dyn/cm² WSS and control, 10 μ M ALLN or 50 μ M calpain inhibitor III for 18 hr. Cultures were fixed, stained, and photographed from the side. Scale bar, 100 μ m.



SUPPLEMENTAL FIGURE 2. ECs alone (HUVEC) or ECs transduced with lentiviruses delivering shRNA directed beta 2 microglobulin (sh β 2M), calpain 1 (shCalp1-1, -2, and -3) or calpain 2 (shCalp2-1 and -2) were stimulated with 1 μ M S1P and 5.3 dyn/cm² WSS for 24 hr. *A and B*: Culture extracts were immunoblotted with antibodies against calpain 1, 2 and GAPDH. *C and D*: The invasion densities quantified for each condition from three individual experiments are summarized (mean \pm SD); **P<0.01 vs. sh β 2M treatment.



SUPPLEMENTAL FIGURE 3. ECs were subjected to steady WSS magnitudes of 0 (static control) or 5.3 dyn/cm^2 WSS in the absence or presence of 1 μ M S1P for 0, 2, and 6 hr. The cells were lysed, and proteins were collected as described in MATERIALS AND METHODS. Western blot analysis was performed with an antibody directed against all forms of calpain 1, calpain 2, and calpastatin. Values (mean \pm SD) were derived by performing densitometric analyses from 3 independent experiments.