(panels A and B, bottom images) and anti-VE-cadherin Ab (Panels A and B, upper images). ERM silencing inhibits S1P-induced redistribution of F-actin and VE-cadherin to the cell periphery. Images are representative of three independent experiments.

Supplemental Figure 1. Specificities of individual ERM siRNAs. Ezrin, radixin, and moesin depletion was induced by specific siRNA duplexes and assessed for cross silencing effects by immunoblotting with appropriate Ab, as compared with treatment with nonspecific (ns) siRNA. Immunoblotting with β-tubulin Ab was used as a normalization control. Quantitative analysis of protein expression was performed by scanning densitometry of the membranes and expressed in relative density units (RDU). Results are means ± S.E. for three independent experiments. *, significant difference ($p < 0.05$) when compare with cells treated with ns siRNA.

Supplemental Figure 2. Effects of ezrin, radixin, and moesin depletion on S1P-induced cytoskeletal remodeling. EC grown on glass cover slips were incubated with combinations of siRNAs to ezrin, radixin, and moesin or treated with non-specific siRNA duplex as described in Methods followed by S1P treatment (1μM, 20 min). ECs were subjected to double immunofluorescent staining with Texas Red phalloidin to visualize F-actin (panels A and B, upper images) and anti-pp-MLC Ab (Panels A and B, bottom images). Incubation with combined siRNAs to ezrin and radixin (g, h) inhibits S1P-induced cortical actin ring formation and peripheral MLC phosphorylation compared with control (nsRNA) incubation (*c, d, arrows*). In contrast, combination siRNA to ezrin and moesin enhances S1P-stimulated cortical actin ring formation and peripheral MLC phosphorylation (*o, p arrows*) compared with incubation with nsRNA. Bar = 10 μM. Results are representative of three independent experiments.

Supplemental Figure 3. Effects of S1P on phosphorylation of PKC isoforms in HPAEC. Confluent HPAEC were treated either with control vehicle or S1P (1 μM) for the indicated times, and phosphorylated PKCζ (A), PKCθ (B), PKCβ (C), and PKCδ (D) were detected via immunoblot. Bar graphs represent relative

densitometry of fold changes in phosphorylated PKC isoforms after S1P relative to vehicle-treated control and expressed as means ± S.E. from three independent experiments. *, significantly different from cells treated with EBM-2 ($p < 0.05$); #, significantly different from cells treated with EBM-2 ($p < 0.01$).

Supplemental Figure 4. Depletion of PKC isoforms and p38 MAPK by siRNA. PKCβ (A), PKCδ (B), PKCθ (C), PKCζ (D), and p38 MAPK (E) depletion were induced by specific siRNA duplexes and assessed for silencing effects by immunoblotting with appropriate Ab, as compared with treatment with nonspecific (ns) siRNA. Immunoblotting with β-actin Ab was used as a normalization control. Quantitative analysis of protein expression was performed by scanning densitometry and expressed in relative density units (RDU). Results are means ± S.E. for three independent experiments. #, significant difference ($p < 0.01$) when compared with cells treated with ns siRNA.

TABLE 1. MotifScan results for ezrin T567, radixin T564, and moesin T557 sites

Protein	*PKC isoform* [a]	*Site*	*Score* [b]	*Percentile* [c]
Ezrin	α, β, γ	T566	0.5410	3.449
	ζ	T566	0.5566	1.407
Radixin	α, β, γ	T564	0.5410	3.449
	ζ	T564	0.5185	0.619
Moesin	α, β, γ	T557	0.5410	3.449
	ζ	T557	0.5566	1.407

[a] Predicted Ser/Thr kinases for ezrin T567 phosphorylation site.

[b] The scan scores start at 0.000 if the sequence optimally matches a given motif and the scores increase for sequences as they diverge from the optimal match. Lower scores in the output are thus better matches.

[c] The percentile ranking of ezrin T567 phosphorylation site in respect to all potential motifs in vertebrate proteins in Swiss-Prot.