Supplementary Figures

FIGURE S1. LPA and S1P activate ERK in adherent microvessel endothelial cells. Serum starved MVECs were treated with 20 μM LPA (A) or 1 μM S1P (B) for the times indicated. Cells were treated with increasing concentrations of LPA (C) or S1P (D) for 6 min. Cell lysates were prepared and immunoblotted for changes in ERK activation. Membranes were stripped and reprobed with antibodies directed against ERK2 to demonstrate equal loading.

FIGURE S2. **Inhibition of ERK activation blocks lysophospholipid-mediated MVEC proliferation.** Serum starved MVECs were treated with 5 μM U0126 for 30 min prior to addition of 20 μM LPA. [^{3}H]-thymidine incorporation (A) as an indicator of cell proliferation was determined as described in Materials and Methods. Statistical differences were measured by comparison with cells in the absence of the inhibitor. $^{**}p<0.01$; $^{***}p<0.001$. In panel B, serum starved cells were treated with U0126 as indicated for 30 min prior to stimulation with either 20 μM LPA or 1 μM S1P for 6 min. Cell lysates were subjected to SDS-PAGE and immunoblotted for changes in phospho-ERK. Stripped membranes were reprobed with an anti-ERK2 antibody for loading control.

FIGURE S3. Lysophospholipid-mediated ERK activation and proliferation in MVECs is G_i-dependent. Serum starved MVECs were treated with increasing concentrations of pertussis toxin (PTx) as indicated for 4 h. Cells were then incubated with 20 μM LPA or 1 μM S1P for 6 min, lysed and immunoblotted for changes in phospho-ERK (A). Membranes were stripped and reprobed with antibodies directed against ERK2 to demonstrate equal loading. Cells treated with

25 ng/ml PTx overnight were assayed for LPA (20 μM) mediated [^{3}H]-thymidine incorporation (B). Statistical differences were measured by comparison with cells in the absence of PTx. ***p<0.001.

FIGURE S4. Effect of growth factor receptor inhibition on lysophospholipid-mediated ERK activation in MVECs. Serum starved cells were treated with either 5 μM AG1478, an EGF receptor inhibitor (A, B), or 5 μM SU5416, a VEGF receptor inhibitor (C, D), for 30 min prior to stimulation with either 10 ng/ml EGF, 10 ng/ml VEGF, 20 μM LPA, or 1 μM S1P for 6 min. Cell extracts were subjected to immunodetection using phospho-specific antibodies to ERK (A, C). Membranes were stripped and reprobed with antibodies directed against ERK2 as loading control. Alternatively, immunoblots were probed with phospho-tyrosine-specific (pTyr) antibodies (B, D), stripped and reprobed with antibodies against the EGF receptor (B) or the VEGF receptor (D).

FIGURE S5. LPA and S1P activate Akt through a G_i-PI3K-dependent pathway. Serum starved MVECs were treated with 20 μM LPA (A) or 1 μM S1P (B) for various times as indicated. Proteins separated on SDS-PAGE were transferred to nitrocellulose and probed with antibodies directed against phosphorylated Ser473 of Akt and phospho-specific antibodies to ERK. Membranes were stripped and reprobed for ERK2 as loading control. Alternatively, cells were treated with 10 μM LY294002, an inhibitor of PI3K, for 30 min (C) or 1 ng/ml PTx for 20 h (D) prior to stimulation with 20 μM LPA or 1 μM S1P for 6 min. Immunoblots were probed with anti-pS473Akt and anti-pERK antibodies. Membranes were stripped and reprobed with an anti-ERK2 antibody as loading control.

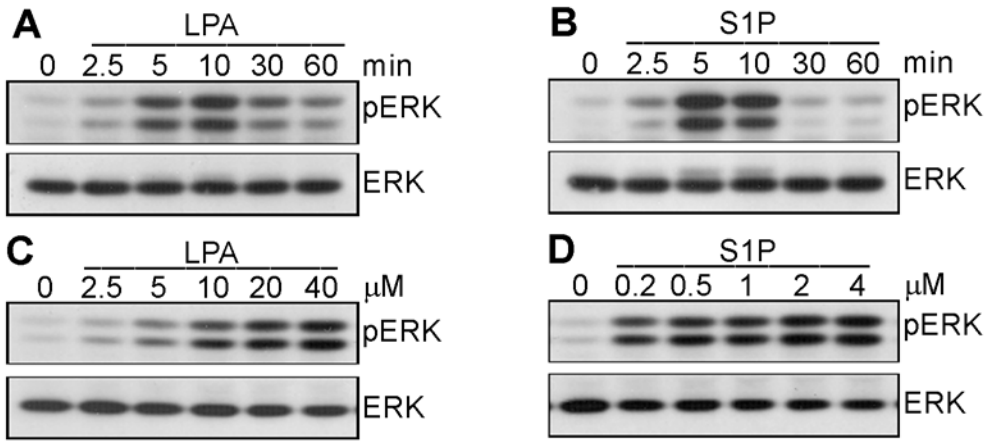

A
LPA
0 2.5 5 10 30 60 min
pERK
ERK
B
S1P
0 2.5 5 10 30 60 min
pERK
ERK
C
LPA
0 2.5 5 10 20 40 μM
pERK
ERK
D
S1P
0 0.2 0.5 1 2 4 μM
pERK
ERK

Figure S1

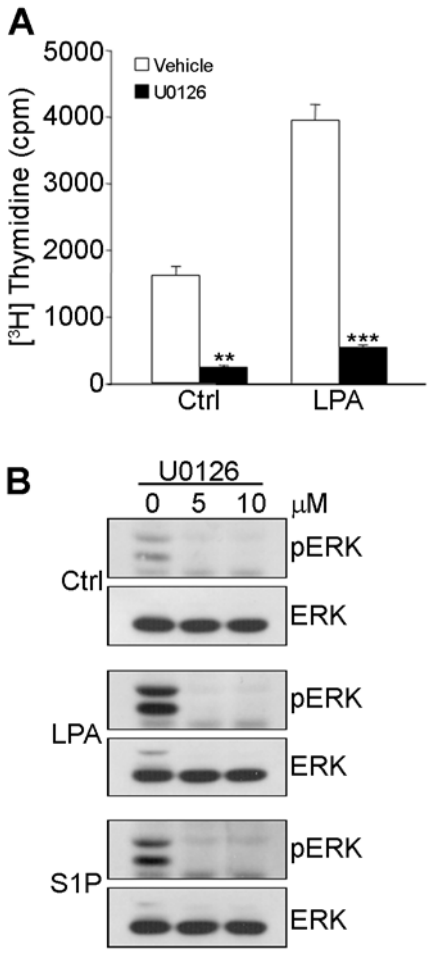
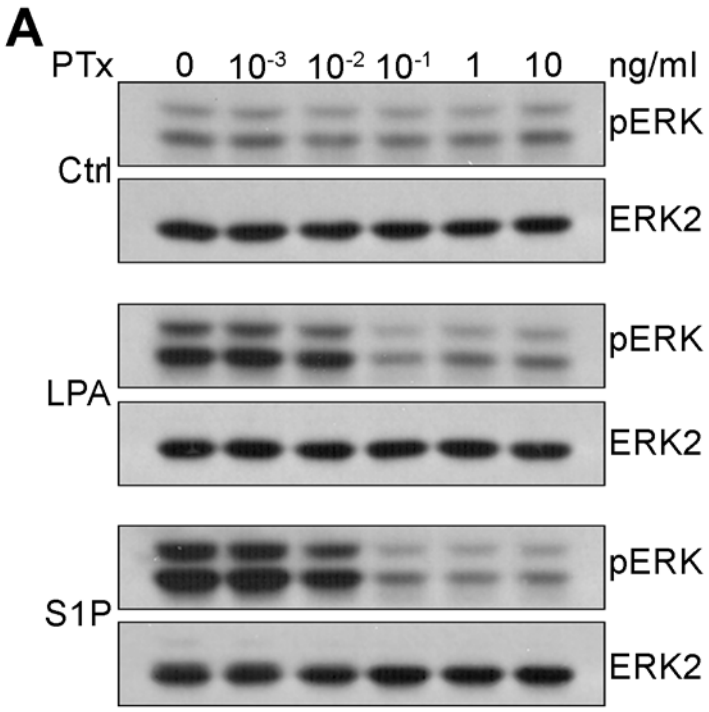

A
5000
4000
3000
2000
1000
0
[3H] Thymidine (cpm)
Vehicle
U0126
**
***
Ctrl
LPA
B
U0126
0 5 10 μM
Ctrl
pERK
ERK
LPA
pERK
ERK
S1P
pERK
ERK

Figure S2


A
PTx
0
10-3
10-2
10-1
1
10
ng/ml
Ctrl
pERK
ERK2
LPA
pERK
ERK2
S1P
pERK
ERK2

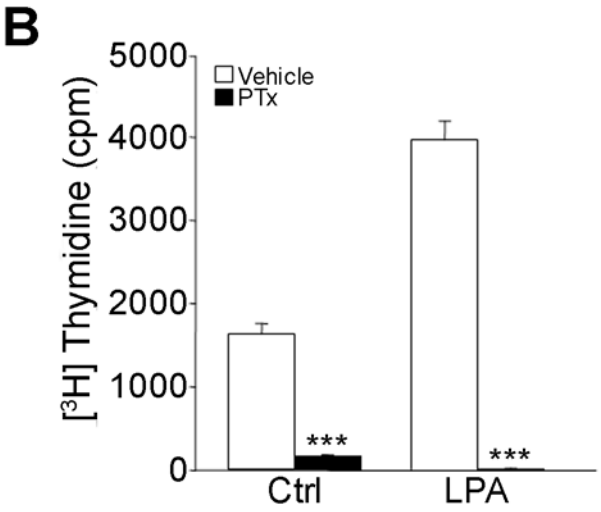

B
[3H] Thymidine (cpm)
5000
4000
3000
2000
1000
0
Vehicle
PTx
***
***
Ctrl
LPA

Figure S3

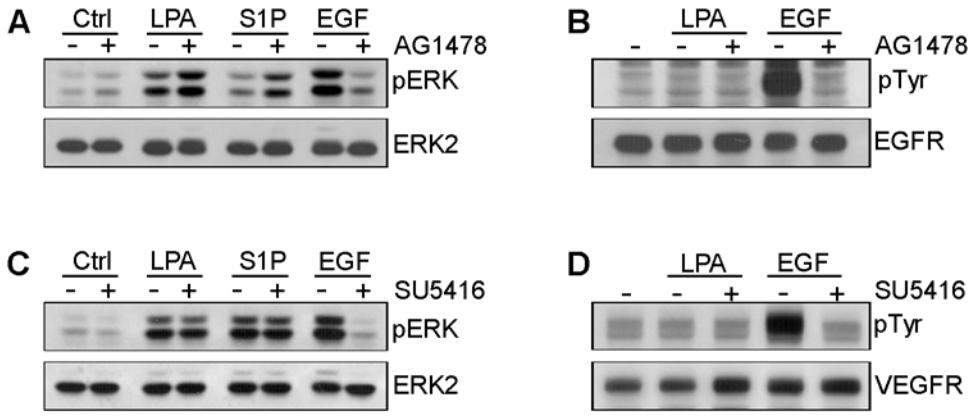

A
Ctrl
LPA
S1P
EGF
- + - + - + - +
AG1478
pERK
ERK2
B
LPA
EGF
- - + - +
AG1478
pTyr
EGFR
C
Ctrl
LPA
S1P
EGF
- + - + - + - +
SU5416
pERK
ERK2
D
LPA
EGF
- - + - +
SU5416
pTyr
VEGFR

Figure S4

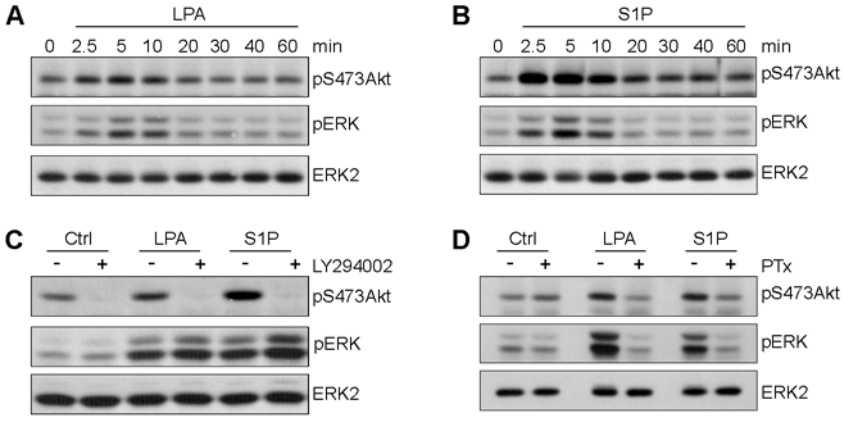

A
LPA
0 2.5 5 10 20 30 40 60 min
pS473Akt
pERK
ERK2
B
S1P
0 2.5 5 10 20 30 40 60 min
pS473Akt
pERK
ERK2
C
Ctrl
LPA
S1P
- + - + - +
LY294002
pS473Akt
pERK
ERK2
D
Ctrl
LPA
S1P
- + - + - +
PTx
pS473Akt
pERK
ERK2

Figure S5