SUPPLEMENTAL DATA

Supplemental Figure 1. Whole blood *Sphk* activity in irradiated mice. 25 μl of whole blood was subject to *Sphk* activity with 20 μmol/L sphingosine and 10 μCi of ^{32}P-ATP diluted in 500 μmol/L ATP for 30 minutes at 37 $^{\circ}$C as described [1]. Note Significant increase in *Sphk* activity Day 1 and Day 3 after irradiation was observed while on Day 10 there was reduction in Sphk activity. Results are average of 3 mice on duplicate samples.

Supplemental Figure 2. *Sphk* activity in liver is stimulated upon transduction with Adeno-*Sphk*1. *Adeno-GFP* and *Adeno-Sphk1* virus was given I.V. as described in Methods. After 72 hours, liver, kidney, lung and spleen were collected and *Sphk* activity was determined as described [1]. *Sphk* activity for *Adeno-GFP* and *Adeno-Sphk1* transduced animals was 14 ± 4.2 and 396 ± 54.2 pmol/min/mg in liver, 37.5 ± 8.5 and 27.1± 4.8 pmol/min/mg in kidney, 137.4 ± 13.1 and 185.9 ± 31.4 pmol/min/mg in lung, and 200.7± 35.6 vs 327.1 ± 65.7 pmol/min/mg in spleen, respectively. Results are average of 3 experiments on duplicate samples.

Supplemental Figure 3. Sphingosine kinase inhibitor (SKI)-1 blocks formation of S1P and release of S1P in MEEC. Confluent MEEC cultures were incubated in absence and presence of 50 μmol/L SKI-1. Amounts of S1P in the intracellular and extracellular compartments were determined as described in Methods. Results are average of 3 independent analyses.

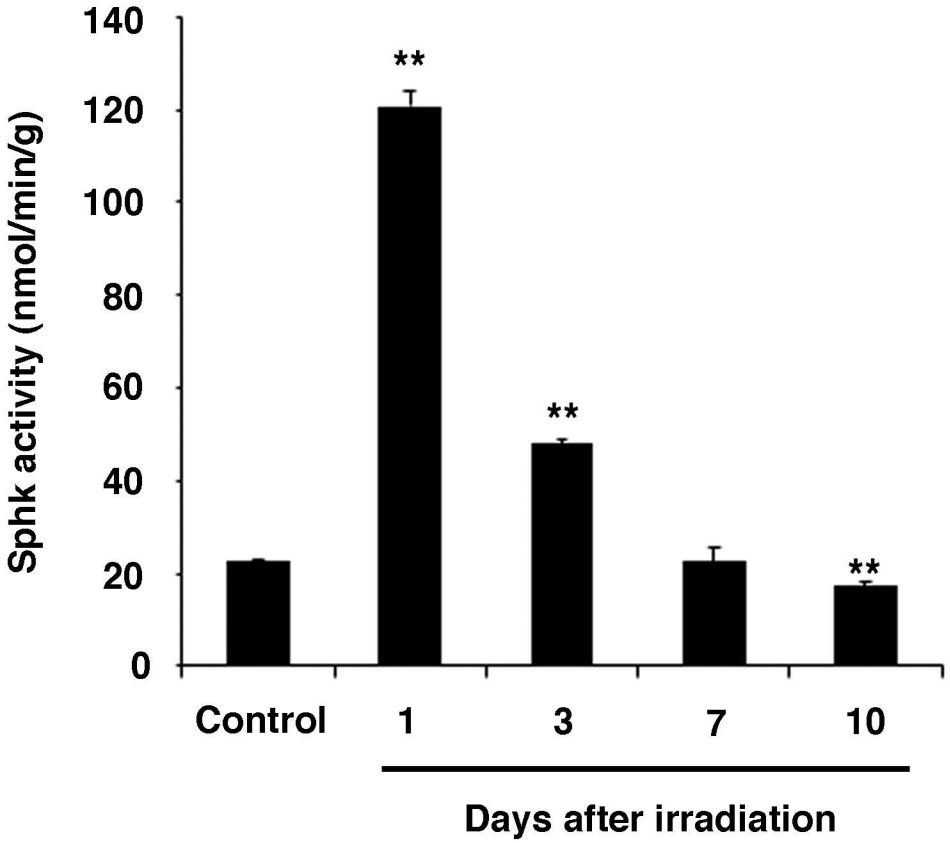
Supplemental Figure 4. MAPK is phosphorylated in MEEC upon laminar shear stress. Confluent MEEC cultures were subject to laminar shear stress. Subsequently immunoblot analysis for MAPK was carried out as described in Methods.

Supplemental Figure 5. Quantitative RT-PCR analysis of S1P metabolizing enzymes

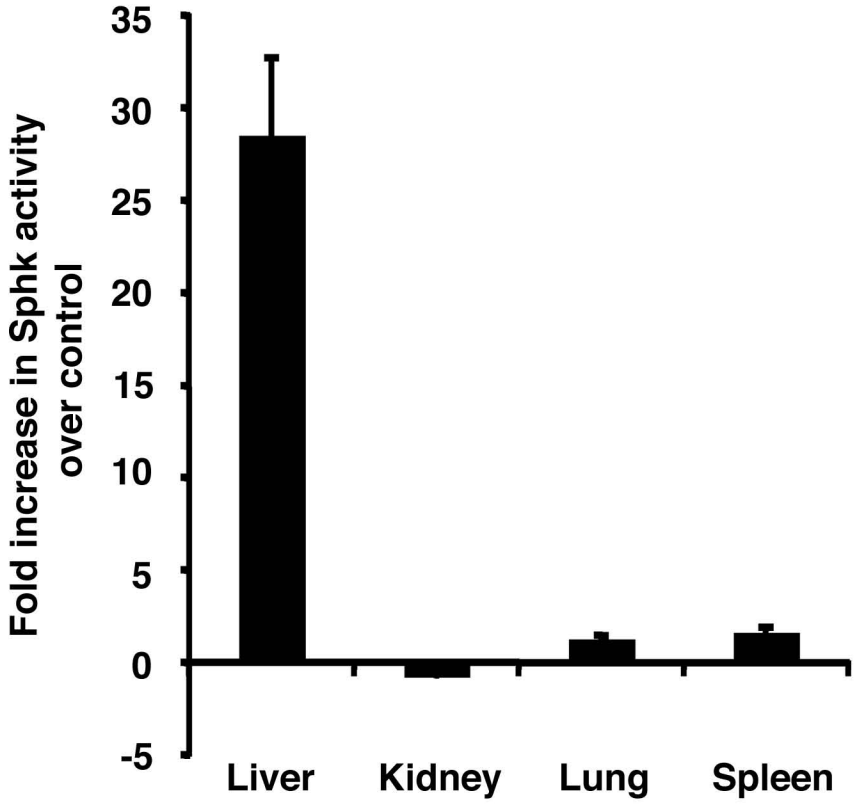
upon shear stress in MEEC. Transcript levels of *Sphk*1, *Sphk*2, S1P *lyase (Sgpl)*, S1P *phosphatase* 1 and 2 (*Sgpp*1 and *Sgpp*2) were determined in static and shear stressed MEEC. Transcripts were normalized to *Gapdh* mRNA. mRNA levels in static control is 100% and mRNA levels in shear stress is presented as % of static control.

REFERENCE

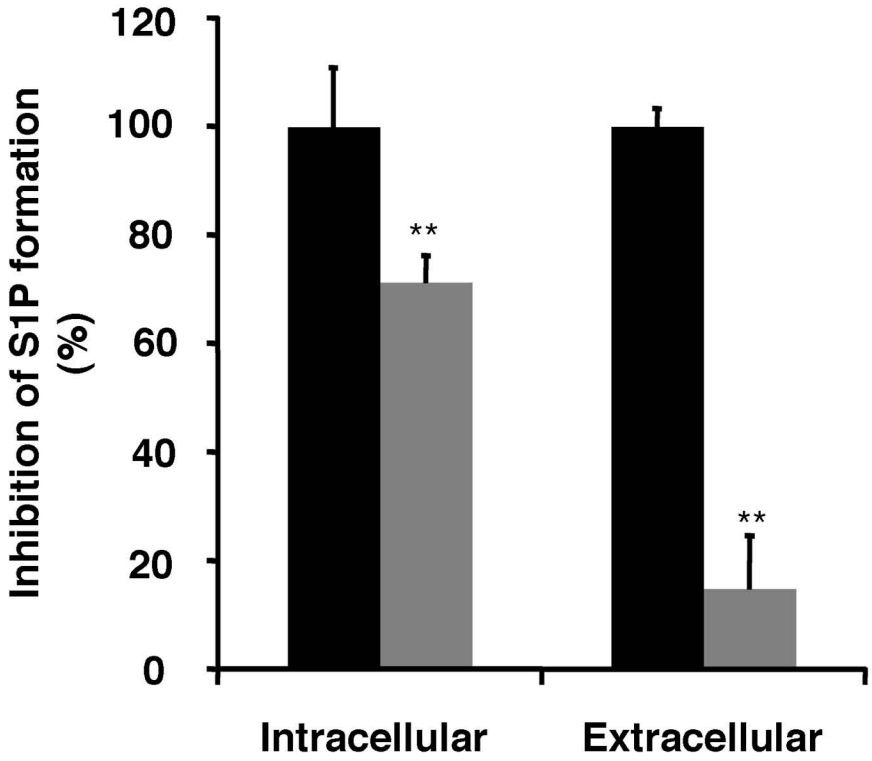
1. Venkataraman K, Thangada S, Michaud J, Oo ML, Ai Y, Lee YM, Wu M, Parikh N, Khan F, Proia RL, Hla T. Extracellular export of Sphingosine Kinase-1a contributes to the vascular S1P gradient. *Biochem J.* 2006;397:461-71.


140
120
100
80
60
40
20
0
Sphk activity (nmol/min/g)
**
**
**
Control
1
3
7
10
Days after irradiation

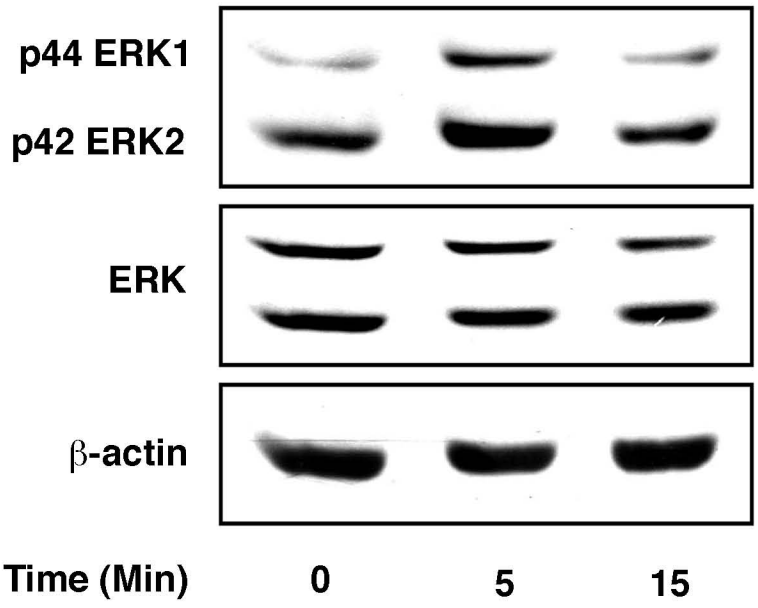
Sup. Fig. 1


Fold increase in Sphk activity over control
35
30
25
20
15
10
5
0
-5
Liver
Kidney
Lung
Spleen

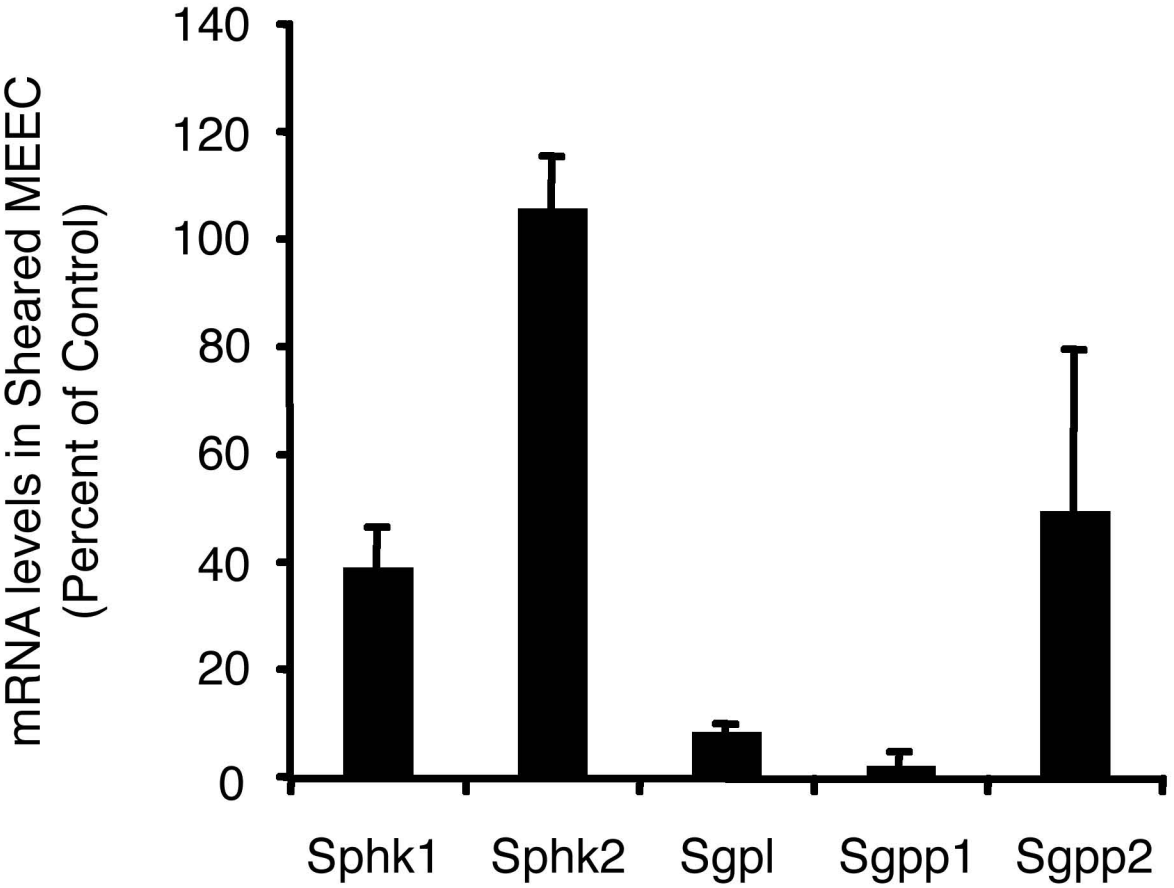
Sup. Fig. 2


Inhibition of S1P formation (%)
120
100
80
60
40
20
0
**
**
Intracellular
Extracellular

Sup. Fig. 3



Sup. Fig. 4


mRNA levels in Sheared MEEC
(Percent of Control)
140
120
100
80
60
40
20
0
Sphk1
Sphk2
Sgpl
Sgpp1
Sgpp2

Sup. Fig. 5