

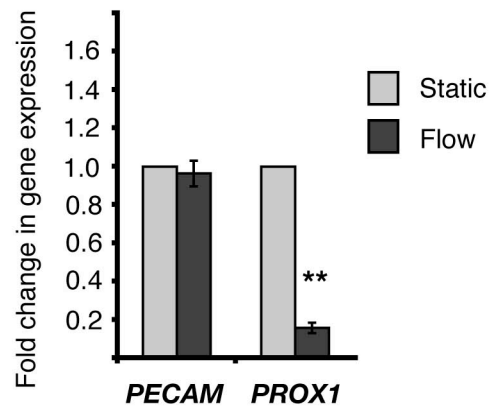
Supplemental Methods

PCR Primer sequences:

Gene	Forward Primer	Reverse Primer
<i>GAPDH</i>	GAAGGTGAAGGTCGGAGTC	GAAGATGGTGATGGGATTTC
<i>PECAM</i>	AACTCAGACGTGCAGTACACGGAA	TTAAGAACCGGCAGCTTAGCCTGA
<i>PROX1</i>	ATAAAGTCCGAGTGCGGCGATCTT	TGACGTGCGTACTTCTCCATCTGA
<i>HEY1</i>	TTTGTCTGAGCTGAGAAGGCTGGT	TCAGATAACGCGCAACTTCTGCCA
<i>HEY2</i>	TAAAGGCTACTTTGACGCACACGC	TTCTGAAGTTGTGGAGAGGCGACA
<i>EFNB2</i>	TGTGCCAGACAAGAGCCATGAAGA	GCATCCTGAAGCAATCCCTGCAAA

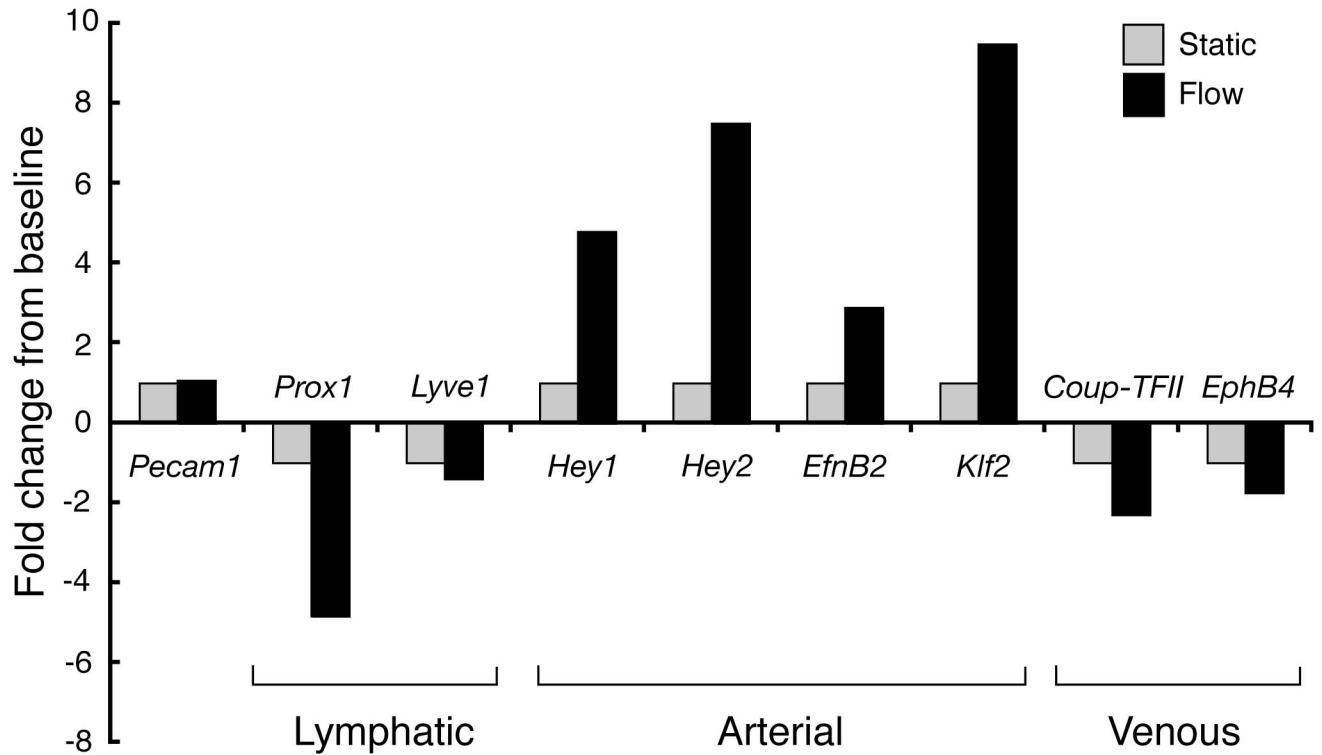
Supplemental Figures and Legends

Supplemental Figure 1



Supplemental Figure 1. *PROX1* expression is down-regulated in LECs exposed to steady flow. LECs were subjected to a shear stress of 20 dynes/cm² using steady flow for 8 hours and the expression of the indicated mRNAs measured using qPCR. N=3. ** p<0.05.

Supplemental Figure 2



Supplemental Figure 2. Up-regulation of arterial genes and *KLF2* in lymphatic endothelial cells exposed to flow. LECs were subjected to a shear stress of 20 dynes/cm² using steady flow for 8 hours and the expression of the indicated mRNAs measured using qPCR. The result shown is representative of 3 individual experiments.

Supplemental Movies 1 and 2. First-pass angiography reveals minimal flow in the blood-filled lymphatics of SLP-76-deficient radiation chimeras. Real-time video microscopy of the intestinal vasculature was performed on lethally irradiated wild-type mice reconstituted with either wild-type (Supp. Movie 1) or SLP-76-deficient (Supp. Movie 2) bone marrow following injection of FITC-dextran into the jugular vein. In wild-type reconstituted animals sequential arterial and venous filling are observed (Supp. Movie 1 and (10)). In animals reconstituted with SLP-76-deficient bone first pass angiography also reveals sequential, rapid filling of the mesenteric artery and vein but only a minimal amount of FITC-dextran passes into the blood-filled mesenteric lymphatic (Supp. Movie 2), indicating a very low level of blood flow. In contrast, in the SLP-76-deficient animal arterial FITC-dextran does not wash out prior to simultaneous rapid filling of the mesenteric vein and lymphatic (compare to Supp. Movies S3 and S4 in (10)).

Supplementary Table 1. Hemodynamic measurements in mesenteric vessels of *Slp-76^{+/+}* and *Slp-76^{-/-}* animals.

	Artery			Vein/Shunt Vessel			Shunt Vessel		
	V_{MEAN} (mm/s)	D (mm)	Shear stress (dynes/cm ²)	V_{MEAN} (mm/s)	D (mm)	Shear stress (dynes/cm ²)	V_{MEAN} (mm/s)	D (mm)	Shear stress (dynes/cm ²)
WT 1	55.3	0.15	103.2	20.4	0.26	22.0			
WT 2	107.7	0.16	188.4	31.7	0.34	26.1			
WT 3	35.5	0.10	99.4	10.6	0.17	17.5			
WT 4	40.0	0.09	124.4	23.2	0.19	34.2			
WT 5	37.8	0.11	96.3	13.8	0.14	27.7			
WT 6	10.3	0.04	71.8	6.6	0.06	30.9			
KO 1	34.6	0.07	138.2	10.8	0.08	37.9	9.8	0.12	22.9
KO 2	17.6	0.06	81.9	13.5	0.13	29.1	19	0.13	40.9
KO 3	67.7	0.07	270.8	5.6	0.18	8.7	5.5	0.14	11
KO 4	10.2	0.08	35.6	6.7	0.19	9.8	5.5	0.16	9.6
KO 5	42.7	0.07	171.0	17.6	0.17	28.9	0	0.19	0
KO 6	24.6			12.9	0.10	36.0	10.3	0.10	28.8
KO 7	16.2	0.06	75.7	8.8	0.14	17.6	8.8	0.13	18.9
KO 8	82.4	0.18	128.2	7.6	0.22	9.7	7.6	0.20	10.7
KO 9	89.2	0.16	156.2	21.2	0.21	28.3	10.4	0.15	19.5