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MATERIALS AND METHODS

Cardiac MRI Studies

Mice were anesthetized with a 1% isofluorane/air mixture administered via a nose cone at 37 ± 1°C. The surface electrocardiogram (ECG) was monitored and the R-wave used to synchronize MR data acquisition. All images were generated on 4.7T horizontal bore spectrometer using an elliptical surface coil (2.5 3.5 cm) for transmission and reception. ECG-gated cine images along the short axis of the heart were acquired using a fast gradient echo sequence that allows for multiple-slices (N_s) and segmented *k*-space (N_{pe} >1) acquisition at multiple time points (N_{ti}). MR parameters used were as follows: repetition time = 12 ms; time of echo = 2.3 ms; field of view = 3.0 cm; matrix = 128 by 128; slice thickness = 1.0 mm, N_{pe} = 1-2, $N_{t\ i}$= 12, N_s = 1 and *Averages* = 8-16. Ejection fraction and cardiac output were determined using the Image Browser program. To calculate stroke volume (SV) the endocardial border of the LV on each slice was defined and LV lumen volume at end diastole (LVEDV) and end systole (LVESV) were obtained using Simpson's rule. SV was determined by subtracting LVESV from LVEDV and cardiac output determined by multiplying stroke volume and average heart rate.

Vascular Physiology Studies

Microsphere studies. 15 μM fluorescent microspheres (0.2 ml, Molecular Probes) were injected into the descending thoracic aorta of anesthetized, ventilated adult mice just above the diaphragm. To increase perfusion of the gut the abdominal aorta was cross-clamped below the level of the renal arteries. To reduce hepatic arterial flow the mice were fasted overnight prior to the study. Microsphere distribution was analyzed with a fluorescent dissection scope two minutes after injection or real time using video microscopy. Neonatal mice were studied immediately after sacrifice by injection of 0.05 ml of microspheres or FITC-dextran (m.w. 2000, 100 mg/kg) into the left ventricle.

*FITC-dextran angiography.*Angiography of neonates was performed immediately after sacrifice by injection of 0.05 ml FITC-dextran (m.w. 2,000,000, 100 mg/kg) into the left ventricle.

Adult studies were performed on live mice anesthetized with a 1% isofluorane/air mixture administered via an endotracheal catheter and temperature maintained at 37 ± 1°C. The right jugular vein was exposed and cannulated with P10 tubing. 0.2 ml of FITC-dextran was then injected intravenously and fluorescent video microscopy used to image the perfusion of the exposed mesentery and gut wall during the first pass of FITC-dextran through the intestinal circulation.

Histology, Immunohistochemistry, and in Situ Hybridization

Immunohistochemistry and in situ hybridization were performed on paraffin-embedded sections as described at www.uphs.upenn.edu/mcrc/histology/histologyhome.html. Antibodies used were MEC 13.3 anti-mouse CD31 (PharMingen) and anti-LYVE(*1*) and RAM34 anti-mouse CD34 (PharMingen) at final concentrations of 1:500, 1:1000 and 1:50 respectively. Anti-sense in situ hybridization probes corresponded to the following cDNA sequences: Prox1: 1702-2153 GenBank accession NM_008937; LYVE: 613-1093 GenBank accession AJ311501; SLP-76: 1069-1546 GenBank accession U20159. TUNEL assays were performed using the cell death detection kit (Roche) as described at www.uphs.upenn.edu/mcrc/histology/histologyhome.html.

Bone Marrow Reconstitution Studies

Bone marrow reconstitution studies were performed as previously described (*S1*) except for the use of 1200 rad of total body irradiation. Mice were studied between 9 and 12 weeks post-transplantation.

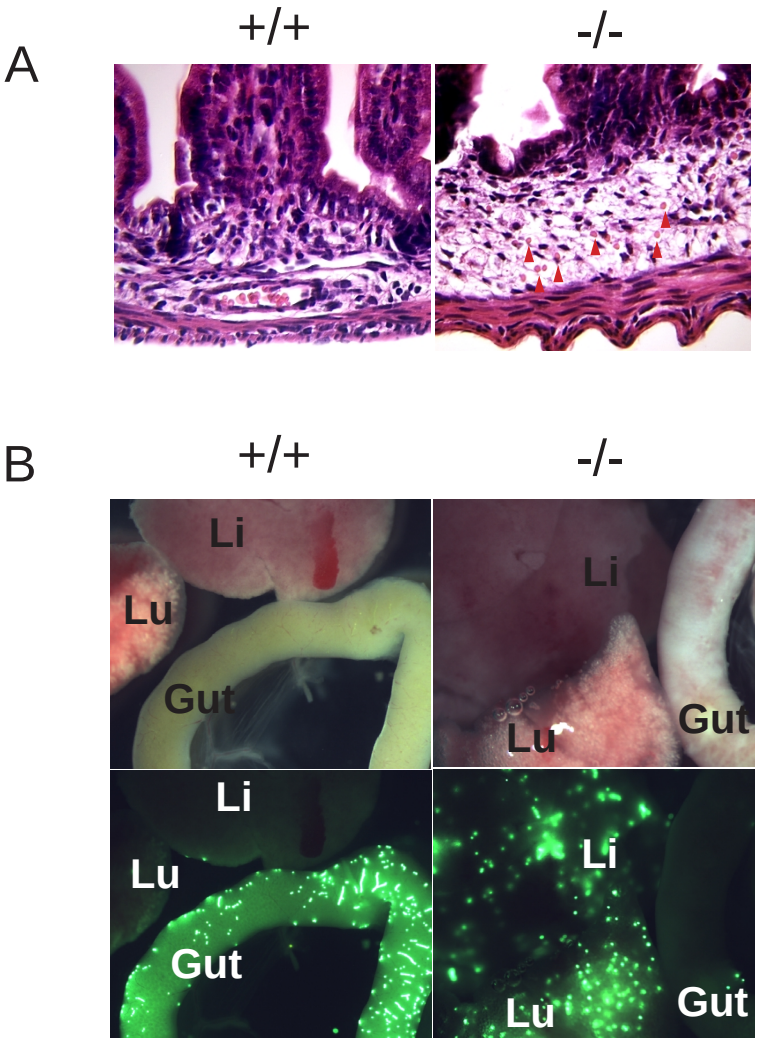

Fig. S1. Development of the A-V shunt in neonatal *slp-76*$^{-/-}$ mice is due to replacement of the normal submucosal blood vessels by a large vascular plexus. (**A**) The small intestine of *slp-76*$^{-/-}$ mice has an expanded submucosa with scattered erthrocytes (red arrowheads) and lacks distinct blood vessels. In the small intestine of a wild-type littermate (+/+) erythrocytes are restricted to the lumen of a thick-walled blood vessel in the submucosa. **(B)** Microsphere injection reveals shunting in association with the replacement of normal submucosal blood vessels by a vascular plexus in *slp-76*$^{-/-}$ mice. Microspheres injected into the left ventricle of wild-type mice (+/+) lodge in the small vessels of the small intestine but do not pass to the liver (Li) or lung (Lu). Microspheres injected into a severely affected *slp-76*$^{-/-}$ mouse (right, -/-) pass through the small intestine nearly completely and lodge in the microvasculature of the liver and lung.

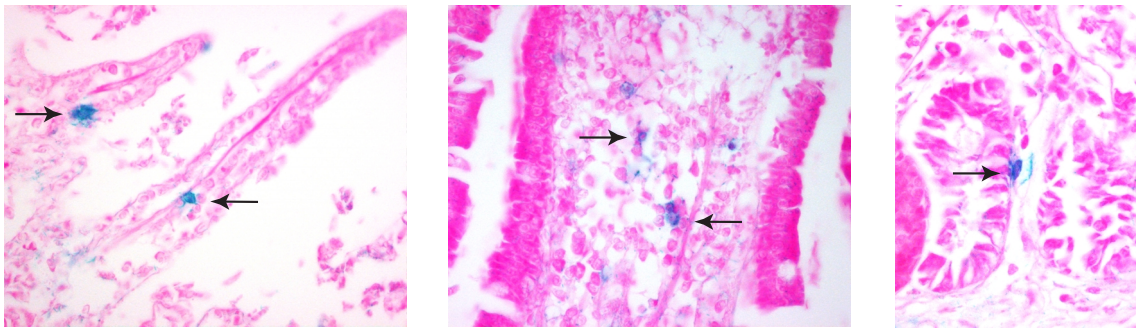

Fig. S2. X-gal staining of the intestine of mice transplanted with Tie2-Lac Z+, *slp-76*$^{-/-}$ marrow does not reveal bone marrow-derived endothelial cells. 40 sections of X-gal stained intestine were examined microscopically to detect blue donor-derived cells that express Tie2-Lac Z. Blue circulating cells could be identified in most sections in the villi where blood-filled lacteals trapped and held large numbers of circulating cells (left and middle panels). Only a single blue endothelial cell could be identified (right panel).

Supplemental Movies 1 and 2. Cardiac MRI reveals normal heart function in *slp-76*$^{-/-}$ mice. ECG-gated transverse images were obtained at the level of the papillary muscles and collated to create movie of a wild-type (Supplemental Movie S1) and *slp-76*$^{-/-}$ (Supplemental Movie S2) heart function during a single cardiac cycle.

Supplemental Movies 3 and 4. First pass angiography of live wild-type (movie S3) and SLP-76–deficient (movie S4) mice reveals vascular continuity between blood and lymphatic vessels and early venous filling in SLP-76–deficient animals consistent with shunting. Real-time video microscopy of the intestinal vasculature was performed following injection of FITC-dextran into the jugular vein. Note the simultaneous filling of both vein and lymphatic following arterial filling in the SLP-76–deficient animal and the failure of arterial FITC-dextran to wash out prior to venous and lymphatic filling.

Supplemental References

S1. B. A. Judd *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **97**, 12056 (2000).