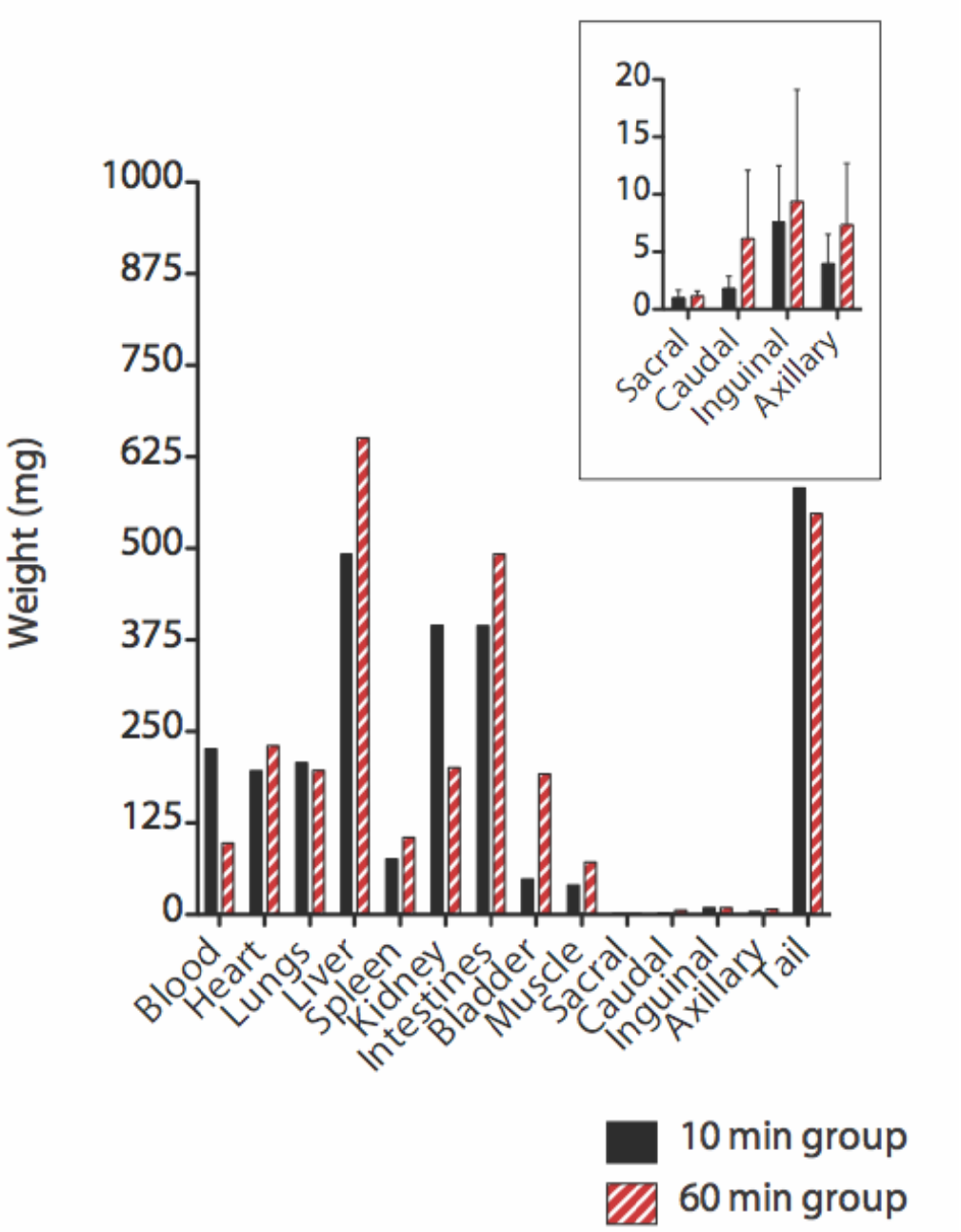
SUPPLEMENTAL METHODS

Detailed Immunohistological Procedures.

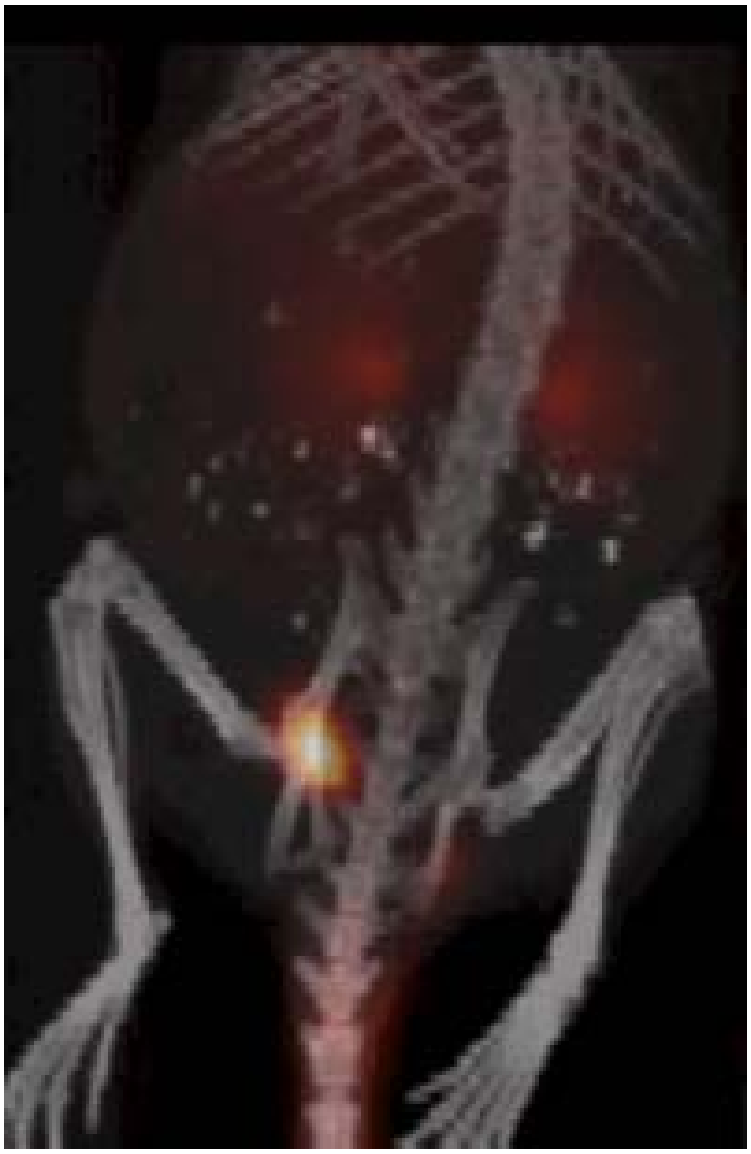
Tissues were fixed in 4% paraformaldehyde (Electron Microscopy Sciences) for 12 h at 4° C, dehydrated in an ethanol gradient, cleared in Histoclear (National Diagnostics) and embedded in paraffin. Sections (5 µm) were deparaffinized, rehydrated and permeabilized. Immunohistochemical staining was performed at the Molecular Cytology Core Facility (MCCF) of MSKCC using the Discovery XT processor (Ventana Medical Systems). Sections were blocked for 30 min in 10% normal goat serum in 0.2% BSA/PBS, incubated for 4 h with 1 µg/mL of the anti-podoplanin primary antibody (Abcam cat. no. ab11936, lot no. 796087) and for 60 min with biotinylated goat anti-rabbit IgG (Vector labs, cat. no. PK6101; 1:200 dilution). Detection was performed with DAB-MAP kit (Ventana Medical Systems) and hematoxylin counterstain. Micrographs were acquired using an Olympus BX40 microscope (Olympus America Inc.) equipped with a motorized stage (Prior Scientific Instruments Ltd.). Images were prepared using ImageJ (NIH) and MosaicJ (Phillipe Thévenaz, Biomedical Imaging Group, Swiss Federal Institute of Technology Lausanne).

Hind Limb Injection of ^{18}F-FDG for Popliteal Node Imaging Delineation.

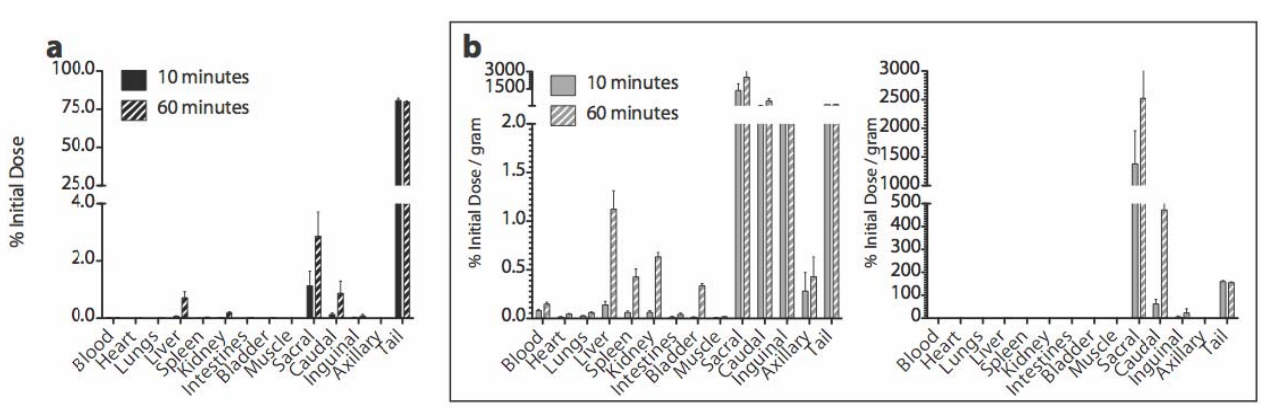
For the dermal foot injection of ^{18}F-FDG the right leg was extended from the body when the mouse was placed in the co-registration restraint device. Three animals were imaged. A dose of approximately 2.8-3.7 MBq (75-100 µCi) in 25 µL was injected into the dorsal skin of the right hind foot. PET/CT imaging was performed as described. Dynamic images (30 s frames) were MAP reconstructed and fused with the CT acquisition. Again, a 3D volume rendered image was prepared using Amira.



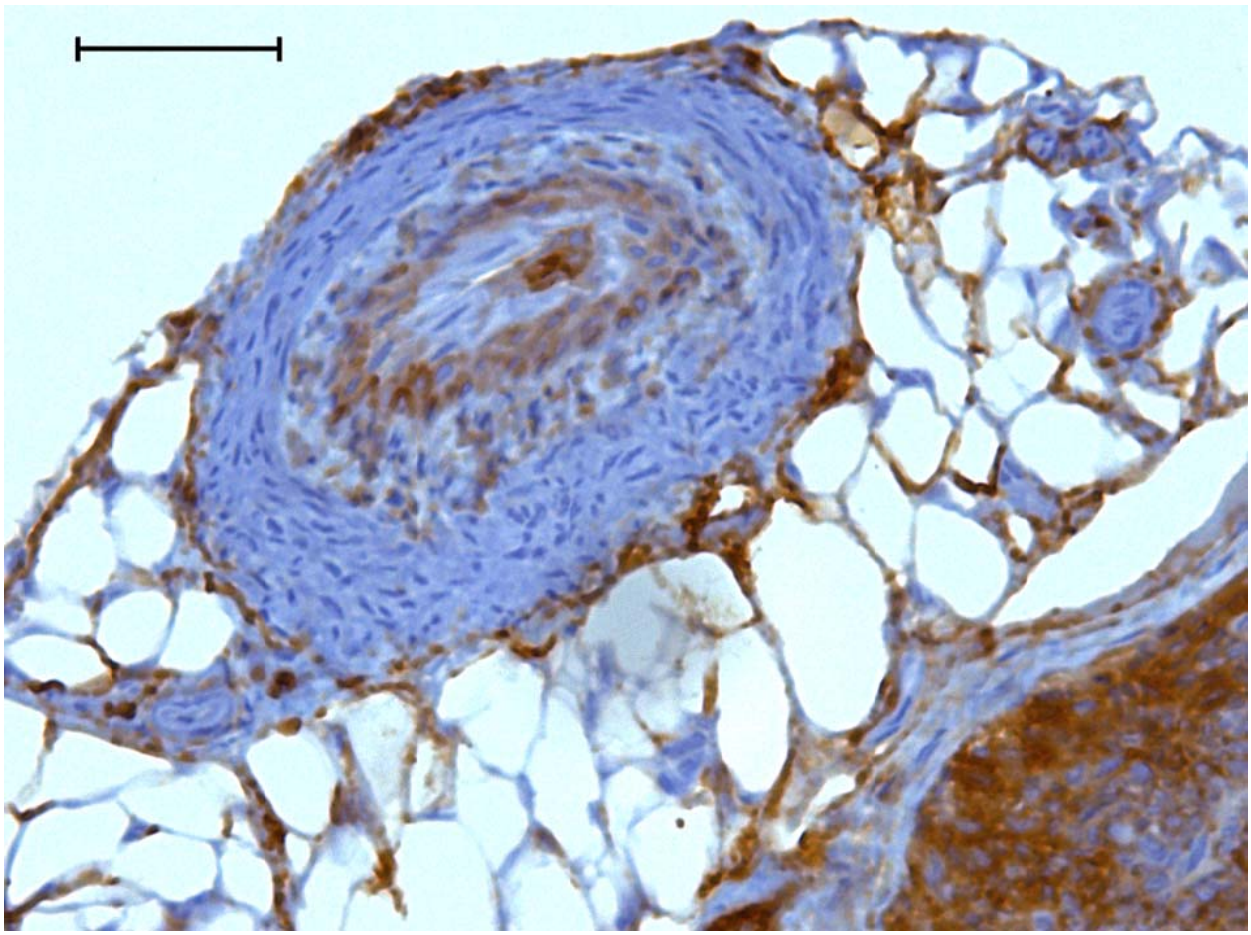
Supplemental Figure 1. Weights of tissues from biodistribution study. Insert of lymphatic tissues (± standard deviation). The light weight of the tissues is an important feature of their high %ID/g biodistribution values. Their small mass and size highlight the need for augmented surgical guidance, particularly in the preclinical sphere.



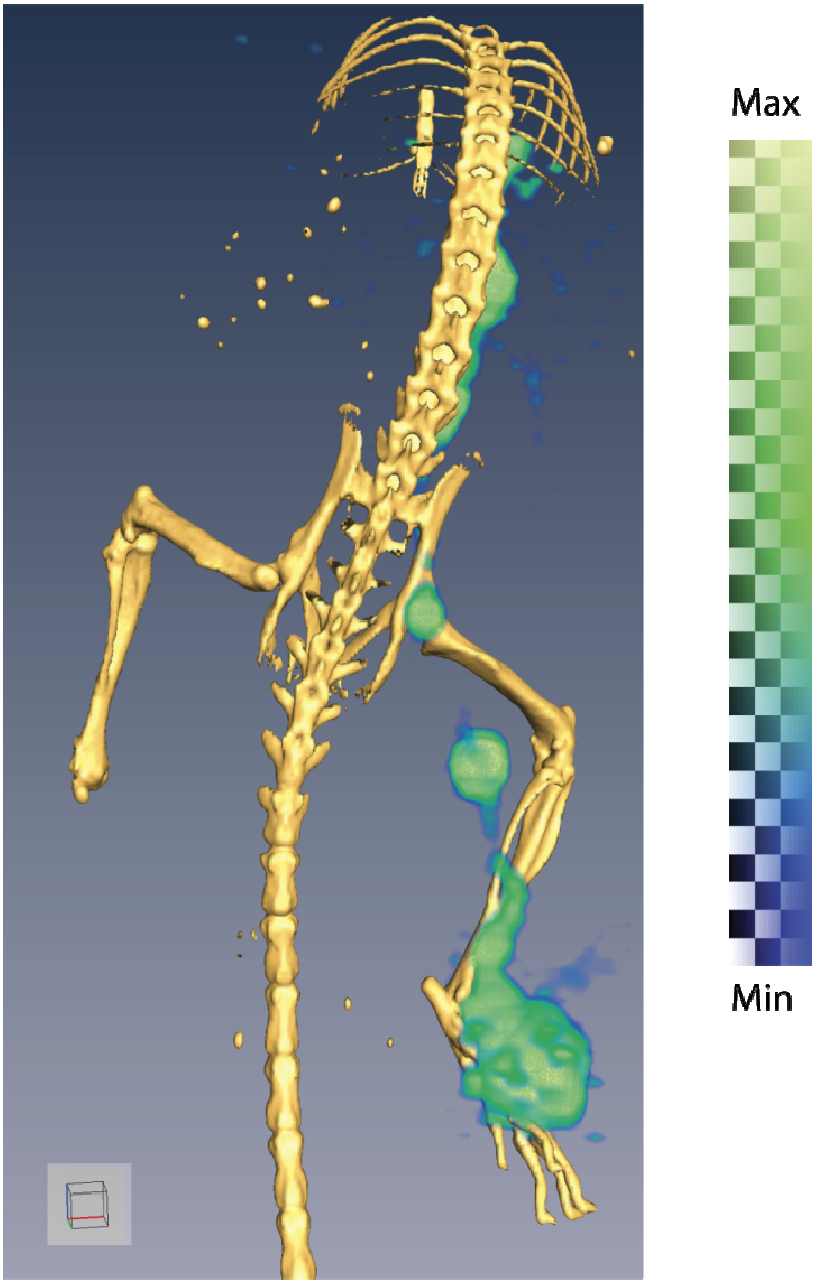
Supplemental Figure 2. Maximum intensity projection (at 30 minutes post injection). The lateral intradermal injection provides uptake preferential to one side of the draining sacral nodes.



Supplemental Figure 3. ^{99m}Tc Sulfur Colloid (^{99m}Tc-SC) biodistribution data, following intradermal injection into the tail. (A) Percent injected dose distribution of the colloid at 10 and 60 minutes demonstrates little of the injected activity clears from the tail. (B) The clinical utility and widespread adoption of this technique are borne out by the high values of nodal uptake on a %ID/g basis.



Supplemental Figure 4. Magnified podoplanin staining of resected lymphatic tissue, as guided by Cerenkov radiation (40x). The node has been axially sectioned and an efferent lymphatic vessel is visible. Here the endothelium of the lymphatic vessel wall is seen surrounded by dense cellularity as counterstained by H&E. Scale bar 50 µm.



Supplemental Figure 5. ^{18}F-FDG lymphography is not limited to mapping the drainage from the tail. A dermal foot injection demonstrates the clear delineation of the draining popliteal and sacral LNs. This representative frame is 1.5 minutes post-injection. 3D-rendered PET data is generated from a weighted average of the intensities from all 2D slices. As such, it is strictly semi-quantitative; the color bar is present to indicate the range of intensities.