SUPPLEMENTARY METHODS

Dual energy-microCT imaging

All DE-microCT scans were performed using prospective cardiorespiratory gating (1) using 40 kVp, 250mA, and 16 ms per exposure for one x-ray source and 80 kVp, 160mA, and 10 ms for the second x-ray source. Both x-ray tubes were triggered simultaneously. ECG was monitored with electrodes (Blue Sensor, Medicotest, UK) taped to the front footpads. Body temperature was maintained at 38°C with heat lamps connected to a rectal probe and feedback controller (Digi-Sense®, Cole Parmer, Chicago, IL). A pneumatic pillow on the thorax was used to monitor respiration. Each acquisition consisted of 300 projections per x-ray source/detector. A single scan required approximately 10 minutes to complete. MicroCT reconstructions were performed with the Feldkamp algorithm (2) and resulted in reconstructed 40 and 80 kVp volumes with isotropic, 88 micron voxels.

Dual Energy Decomposition

Affine registration was performed to improve registration between corresponding 40- and 80-kVp reconstructed volumes using ANTs, an open-source, ITK-based registration toolkit (Advanced Normalization Tools, http://stnava.github.io/ANTs/; svn 1409) (3). Affine registration in ANTs optimized mutual information (4). Tests with subjects scanned consecutively have shown a registration accuracy of half a voxel along each spatial dimension. Registration between each pair of 40- and 80-kVp data generally completed in less than 2 minutes using a Mac OS X workstation with dual 2.66 GHz, quad-core Xeon processors and 16 GB of RAM.

To improve the decomposition, each dataset was denoised using joint bilateral filtration (BF). Joint BF is a spectral extension of the well-characterized edge preserving smoothing filter that considers both the distribution of neighboring voxels in space and intensity. Joint BF was implemented using MATLAB (MathWorks, Natick MA), and generally completed within 15 to 20 minutes per set of energies. Details of the application of joint BF to murine microCT data (5) and a quantitative evaluation of the application of BF to DE-microCT (6) have been described previously.

DE decomposition of gold and iodine was performed after registration and filtration, using corresponding 80- and 40-kVp data by solving the following linear system at each voxel:

$$\mathrm{c} = \mathrm{A}^{-1}\mathrm{b}$$

Expanding the linear system:

$$\begin{bmatrix} \mathrm{c_I} \\ \mathrm{c_{Au}} \end{bmatrix} = \begin{bmatrix} \mathrm{A_{I,40}} & \mathrm{A_{Au,40}} \\ \mathrm{A_{I,80}} & \mathrm{A_{Au,80}} \end{bmatrix}^{-1} \begin{bmatrix} \mathrm{b_{40}} \\ \mathrm{b_{80}} \end{bmatrix}$$

In this formulation, *c* was the least-squares solution for the concentration of the iodine (c_I) and gold (c_{Au}) in mg/mL in the voxel under consideration. *A* was a constant sensitivity matrix measured in Hounsfield units (HU/mg/mL) for iodine ($A_{I,40}$, $A_{I,80}$) and gold ($A_{Au,40}$, $A_{Au,80}$) at 40 and 80 kVp, respectively. Finally, *b* was the intensity of the voxel under consideration at 40 kVp (b_{40}) and 80 kVp (b_{80}) in Hounsfield units. Values for $A_{I,40}$, $A_{I,80}$, $A_{Au,40}$, and $A_{Au,80}$ were determined using a calibration phantom and were 36.36, 53.56, 102.00, and 73.16 HU/mg/mL, respectively (see optimization in (5)). Voxels with negative concentrations of both materials were set to zero. Voxels with a negative concentration of one material and a positive concentration of the other material were projected onto the subspace of positive concentrations. Semi-automated

segmentation with Avizo (Visualization Sciences Group, Burlington, MA) was used to separate bones from the gold and iodine maps. The accumulated mass of Au measured in the Au map post decomposition was used to assess vascular permeability, as described in detail in (5).

4D-microCT imaging

To assess cardiac function we used 4D-microCT with retrospective cardio-respiratory gating (7). Projection images were acquired at 80 kVp, 80 mA, 10 ms per exposure with a rapid and constant rate of 20 projections/second without waiting for cardiac and respiratory coincidence. Respiratory signals and ECG were monitored and saved in synchrony with the acquisition of the projections. Sampling involved 5 rotations of the animal, with 450 projections acquired per each full rotation. A total of 2250 projections were acquired, with an acquisition time between 5 and 10 minutes. The procedures for post-sampling processing and reconstruction based on retrospective gating data have been described in detail elsewhere (7). MicroCT imaging resulted in 10 separate 3D datasets that correspond to 10 phases of the cardiac cycle, each with a matrix size of 512x512x300 and a voxel size of 88 microns.

MicroSPECT imaging

During image acquisition, animals were placed prone on a custom heated animal bed with integrated electrocardiogram (ECG) and respiratory monitoring (MILabs, Utrecht, The Netherlands). Body temperature was maintained at 38°C. Mice were maintained under general anesthesia throughout the imaging procedure with 1.5% isoflurane in mixed gas,

delivered via nose cone at a rate of 0.4 liters/minute. Anesthetized mice were injected via tail vein catheter with 99mTc -tetrafosmin (GE Healthcare, Arlington Heights, IL) prior to image acquisition.

Immediately following radiotracer injection, microSPECT images were acquired over 30 minutes. The field-of-view was adjusted to the margins of the heart using orthogonal radiographs generated by the integrated microCT unit. Data were acquired in listmode and reconstructed with retrospective cardiac gating using the built-in U-SPECTII/CT reconstruction software; this software utilizes the Pixel-based Ordered Subset Expectation Maximization (POSEM) iterative reconstruction algorithm (6 iterations; 16 subsets; 0.125 mm voxel size). Reconstructed images were viewed using PMOD v.3.3 biomedical image quantification software (PMOD Technologies Ltd., Zurich, Switzerland.

REFERENCES

1. Badea C, Hedlund LW, Johnson GA. Micro-CT with respiratory and cardiac gating. *Med Phys* 2004;31:3324-3329.
2. Feldkamp LA, Davis LC, Kress JW. Practical cone-beam algorithm. *J. Opt. Soc. Am.* 1984;1:612-619.
3. Avants BB, Tustison NJ, Song G*, et al.* A reproducible evaluation of ANTs similarity metric performance in brain image registration. *Neuroimage* 2011;54:2033-2044.
4. Hermosillo G, Chefd'Hotel C, Faugeras O. Variational methods for multimodal image matching. *International Journal of Computer Vision* 2002;50:329-343.
5. Clark DP, Ghaghada K, Moding EJ*, et al.* In vivo characterization of tumor vasculature using iodine and gold nanoparticles and dual energy micro-CT. *Phys Med Biol* 2013;58:1683.
6. Badea CT, Guo X, Clark D*, et al.* Dual-energy micro-CT of the rodent lung. *Am J Physiol Lung Cell Mol Physiol* 2012;302:L1088-1097.
7. Johnston SM, Johnson GA, Badea CT. Temporal and spectral imaging with micro-CT. *Med Phys* 2012;39:4943-4958.