Supplemental materials

Histological procedures

After the last MR/PET imaging session, rats were deeply anesthetized with 5% isoflurane and transcardially perfused with 4% paraformaldehyde (PFA) in phosphate buffer (0.1 M, pH 7.4). Bone specimens were removed and placed in a histological cassette and post fixed overnight at 4°C in Neutral Formalin Buffer (10%). Then, the cassettes were placed into Super Decalcifier II Delicate solution (Polyscience Inc., Warrington, PA, USA) for 72 hours at room temperature. Decalcifying solutions were changed every 24 hours (twice) until complete decalcification was verified with the Poly-NoCal End Point Determination Kit (Polyscience Inc., Warrington, PA, USA). The bones were embedded in paraffin, bone sections (3 μm) were generated and classical hematoxylin-eosin staining was performed. Ki67 immunolabeling was performed on the same samples. After antigen retrieval, H_2O_2 treatment, and blocking of non-specific sites with TBS-BSA (2%), the sections were incubated overnight at 4°C with the anti-Ki67 antibody (1:100; Rabbit, Ki67 [SP6], monoclonal antibody, ab1667; Abcam, Cambridge, MA, USA). The samples were then incubated with HRP-conjugated goat anti-rabbit secondary antibody (Vector Labs, Burlingame, CA, USA). The signal was amplified using the Vectastain ABC Elite kit (Vector Labs, Burlingame, CA, USA) and developed using diamino-benzidene (ImmPACT DAB; Vector Labs, Burlingame, CA, USA) as a chromogen. For negative controls the primary antibody was omitted during the immunohistochemical procedure. Bright-field pictures were taken with a Leica

DM4000 microscope equipped with a Leica DFC350FX camera.

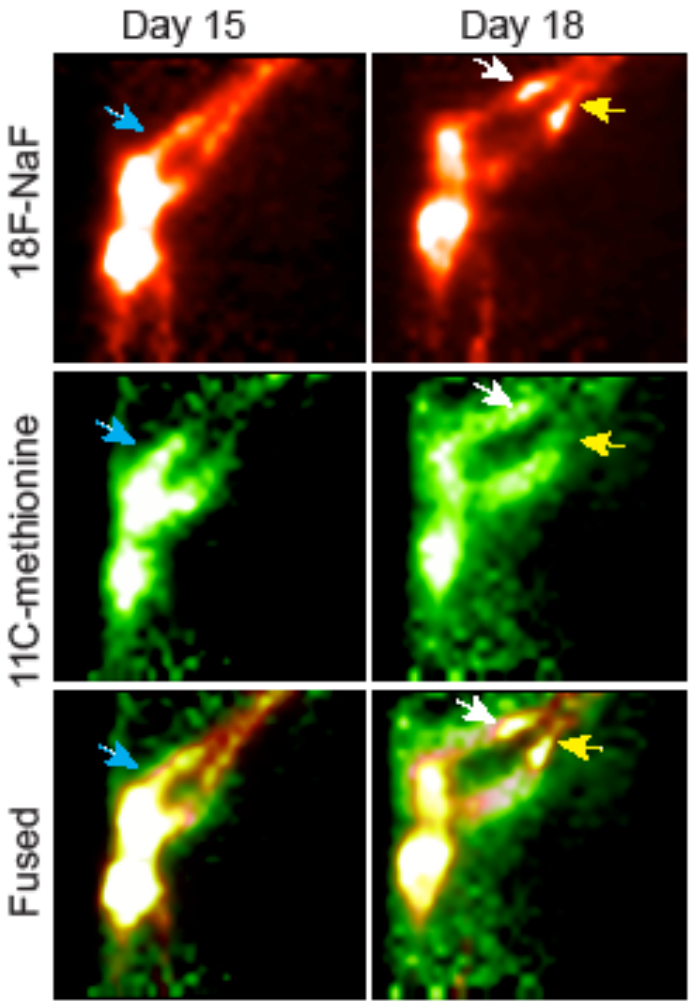
Radiological analysis

Micro-CT scans were performed using a high-resolution Micro-CT scanner (SkyScan-1072, Aartselaar, Belgium), and the subsequent 3D architecture analyses were completed using the associated software applications. The scanner was equipped with a sealed microfocus X-ray tube with a power source ranging from 20 to 100 kV (0 to 250 μA) and an X-ray CCD-camera based on a cooled 12-bit CCD-sensor of 1024 × 1024 pixels. The distal femurs were scanned at a source power of 45 kV/222 μA and a spatial sampling of 14.06 μm/pixel (cross-section image resolution). The rotation was set at 0.9 degrees/step for 180 degrees, and the exposure time was set at 3.0 sec/step. Reconstruction of the dataset from the X-ray shadow images obtained from the scans was performed using the following algorithm parameters: Beam Hardening Correction of 40%, Ring Artifact Correction of 4, Min. for CS to Image Conversion = 0, Max. for CS to Image Conversion = 0.38, Step 1, Top-Bottom 998-25, FOV off and using NRecon (version 1.6.1.3), generating a dataset of cross-sections that was then used to analyze the 3D architectural parameters and create 3D models with the CT-Analyzer (version 1.10.01).

Magnetic Resonance Imaging

MRI studies were conducted at the Centre d'imagerie moléculaire de Sherbrooke (CIMS) with a 210 mm diameter small-animal 7T scanner (Agilent

Technologies Inc., Palo Alto, CA, USA) and a 63 mm diameter volume RF coil. The rats were placed supine in an MRI-compatible cradle equipped with a custom-made paw support designed to position limbs both stably and reproducibly. The animals were anaesthetized with 3% (induction) and 1.5% (stabilization) isoflurane in oxygen. A feedback-controlled animal warm-air heater system (32°C) was used to maintain body temperature at physiological levels, and the respiration rate was continuously monitored (SA Instruments Inc., Stony Brook, NY, USA). The MRI protocol included the acquisition of axial (sagittal) pre-contrast and 10 min post-contrast T1-weighted images using a gradient echo sequence with a TR of 210 ms, TE of 3.35 ms, flip angle of 30^0, matrix of 256 × 256, FOV of 60 × 60 mm^2, NA of 8 (30 sagittal slices), and thickness of 1.5 mm. A 500 μL bolus of the contrast agent (Gd-DPTA, Magnevist, Berlex) was injected in the tail vein. The animals were imaged at day 15 to ascertain the presence of the tumor and at day 18 to perform the analyses.



Supplemental Figure 1. Fusion of ^{11}C-MET and ^{18}F-NaF PET images. Fused PET images showed a strong co-localization of ^{18}F-fluoride and ^{11}C-MET uptake on the anterior surface of the femur on day 15 (blue arrows). On day 18, the ^{11}C-MET uptake was largely exempt of fluoride labeling, except for small fluoride-positive foci on the proximal edge of the ^{11}C-MET-uptaking tumor (white arrows). It seems that the most proximal part of ^{18}F-fluoride-uptaking regions was not co-localized with ^{11}C-MET uptake (yellow arrows).