PET Imaging Post-Processing

CT slices were reconstructed using COBRA (Exxim Computing Corporation) on volumes of 480 x 480 x 683 isotropic voxels, 191.92 μm^3. Each 20 minute PET scan was reconstructed with Micro-Q (Siemens AG), using the OSEM-3D algorithm with the attenuation map obtained from the corresponding reconstructed CT scan. To isolate the 3'-deoxy-3'-^{18}F-fluorothymidine (^{18}F-FLT) activity in the bone marrow (BM) compartment from various regions, a mask corresponding to the bone structures was generated from the CT scan and applied to the PET data (MatLab, The MathWorks, Inc.). The CT volume was thresholded to select for bone regions, and the voids inside the bones corresponding to BM compartments, were filled. The resulting volume was resampled to produce a binary mask with voxels matching those of the PET scan. The mask was imported into OsiriX (open source software, http://www.osirix-viewer.com) where it was refined manually to insure that all desired voxels were included and any extraneous objects, such as food particles in the gastrointestinal tract, were removed; the tail was removed for consistency (Supp. Video 1). In regions where the rat volumes overlap, a cut-off point was selected and the redundant parts were trimmed so that each part of the skeleton remained in only one scan.

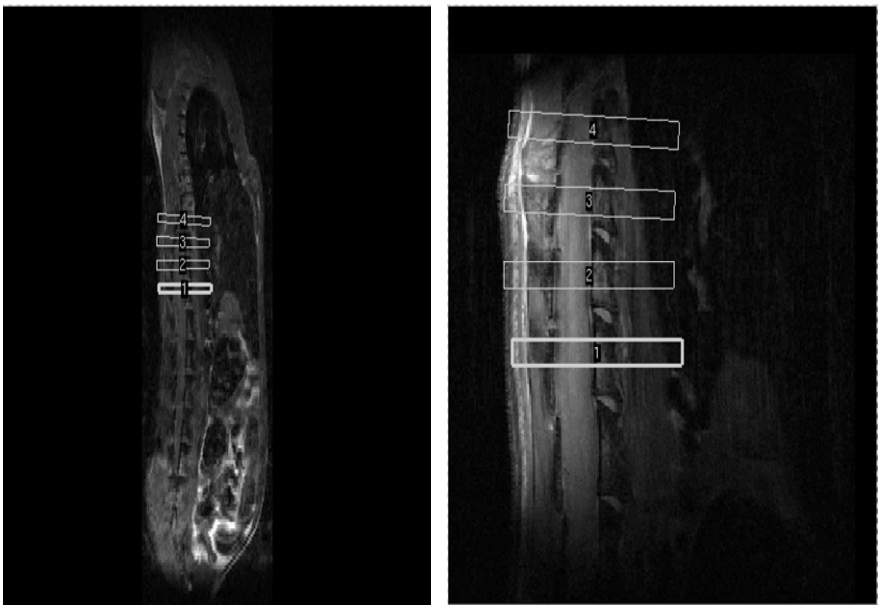
Quantification of Cell Proliferation

Each binary mask refined in OsiriX was exported back to MatLab where it was applied to the corresponding PET volume. The masks were cut into segments corresponding to different parts of the skeleton using the "3D scissors" tool of OsiriX, thus allowing for quantification of radioactivity in different regions. The average activity per unit volume, adjusted to the time of the FLT injection as part of the PET

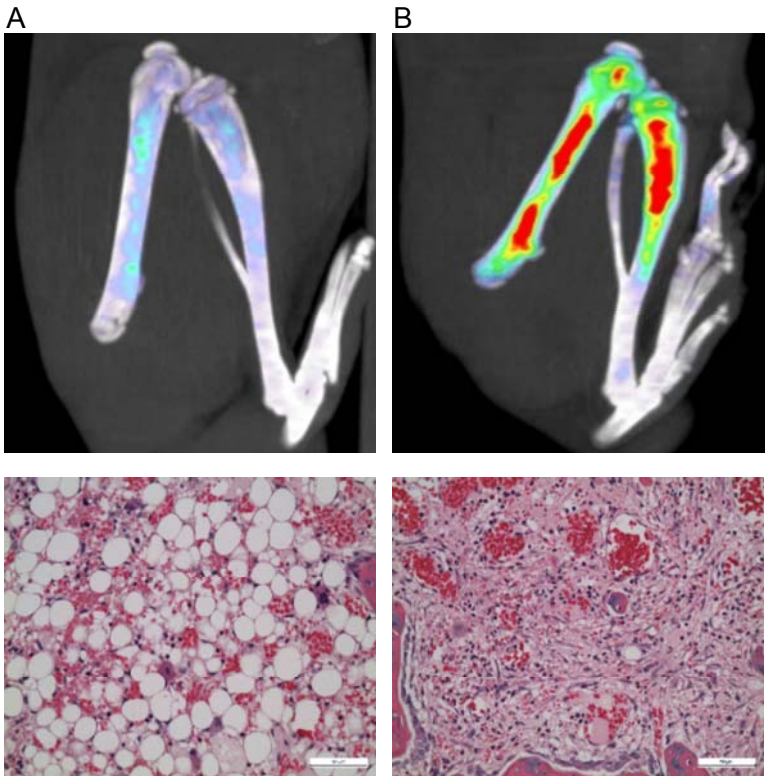
reconstruction, was used to calculate the Standard Uptake Value (SUV) by body weight for the regions studied: SUV_{BW} = average activity in ROI (Bq/ml) * animal weight (g) / injected dose (Bq).

MR Data Analysis

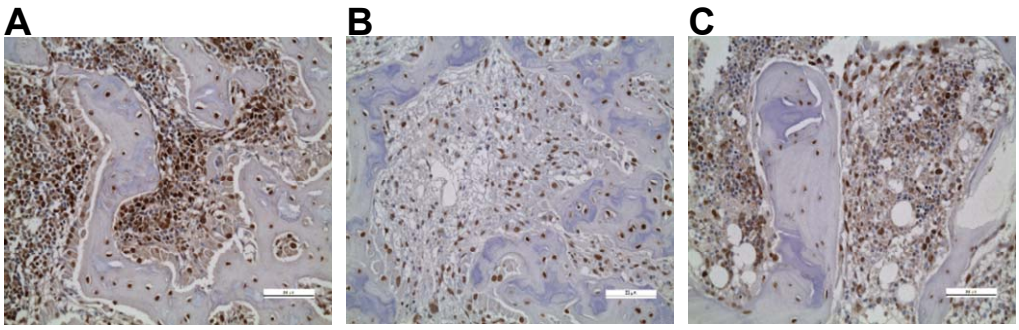
Images were analyzed offline using customized scripts in MatLab. The change in relaxation rate, ΔR_2, color-coded relative blood volume maps were calculated on a voxel-by-voxel basis from spin echo datasets where the signal intensity was measured before (I_{before}) and after (I_{after}) ferumoxytol injection using the formula $\Delta R_2 = -\ln(I_{after}/I_{before})/TE$, where TE is the echo time of the imaging sequence. A region of interest (ROI) was drawn around the BM and mean ΔR_2 were determined.



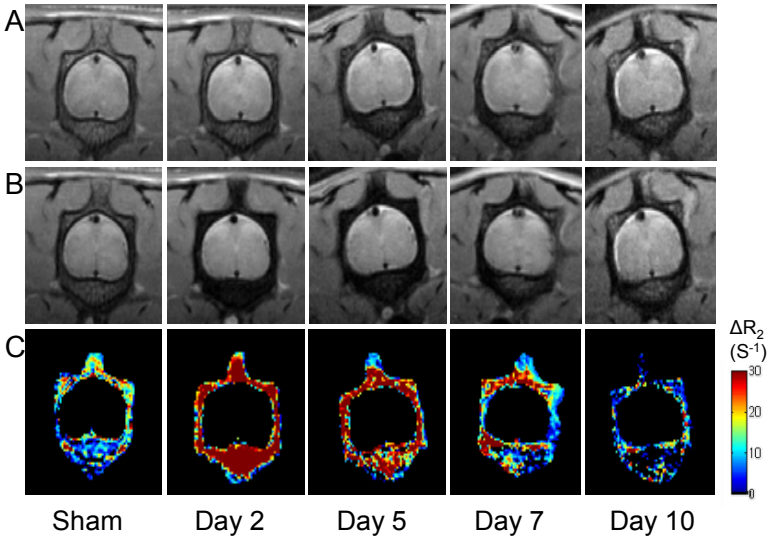
SUPPLEMENTAL FIGURE 1. Images showing axial slices selected for susceptibility contrast MR imaging of bone marrow.



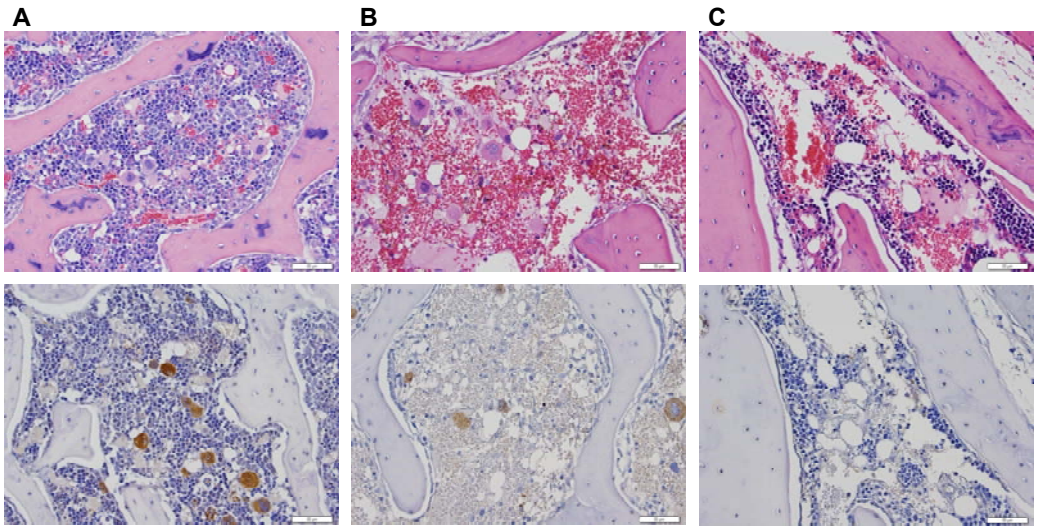
SUPPLEMENTAL FIGURE 2. Proliferation drastically decreases immediately after radiation exposure and recovers at different rates based on the degree of shielding. PET/CT (top) and hematoxylin and eosin (bottom) images of one leg shielding at (A) 2 days and (B) 10 days post irradiation.



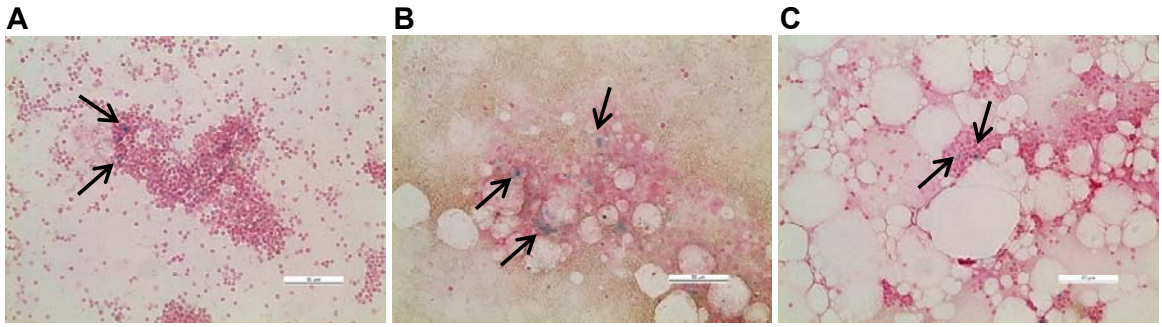
SUPPLEMENTAL FIGURE 3. Bromodeoxyuridine staining confirms our observations from PET data of changes in proliferation caused by radiation exposure. Cross-sectional femurs stained with bromodeoxyuridine (dark brown) from (A) sham irradiation (B) 2 days and (C) 10 days post one leg shielding.



SUPPLEMENTAL FIGURE 4. Mask outlining the region of interest on T_2 weighted images. (A) before injection of ultrasmall superparamagnetic iron oxide particles (B) after injection and (C) the corresponding heat mask of the change in relaxation rate, ΔR_2, at various timepoints post irradiation.



SUPPLEMENTAL FIGURE 5. Immunohistochemical evidence of radiation-induced vascular damage in the exposed region. Hematoxylin and eosin (top) and Factor VIII (bottom) at (A) sham irradiation (B) 2 days and (C) 10 days post half body shielding.



SUPPLEMENTAL FIGURE 6. Immunohistochemical evidence of radiation-induced vascular damage at (A) sham irradiation (B) 2 days and (C) 10 days post half body shielding. Arrows point to positively stained iron particles, not all-inclusive.