Supplemental data

Radiolabeling, cell culturing, and animal model work were performed as previously published by our group (1). In vivo experiments were performed in accordance with permission from the local ethical committee for animal trials (reference number M32-15). Three BALB/cAnNRj mice (Janvier Labs, Le Genest-Saint-Isle, France) injected with 40 MBq 177Lu-PSMA-617 were sacrificed and dissected three days post-injection to collect tumors. These were put in freeze-gel (cryo-mount) and snap-frozen on dry ice. Tumors were cryo-sectioned in 10 µm thick sections in a consecutive series and mounted. Histological hematoxylin and eosin staining (HE-staining) was performed on every second section and imaged on an automated whole slide imager (Carl Zeiss AG, Oberkochen, Germany).

Digital autoradiography (DAR) was performed on a Biomolex 700 Imager (Biomolex AS, Oslo, Norway) on the remaining sections to detect the intra-tumoral distribution of ^{177}Lu activity for a minimum of 1440 minutes. The resulting images were reconstructed and corrected for dead pixels and system activity calibration.

Cell nuclei segmentation

Among the HE images, a representative section with a minimum of folds or cracks was selected for each tumor. To segment the cell nuclei, the HE-images were median-filtered, and regional minima were calculated with the Matlab-function *imextendedmin,* calculating regional minima with the extended-minima transform, which delineated the cell nuclei. The center of each cell nucleus was calculated. To generate a cell density map, a downsized image with a 50 x 50 µm^2 pixel size (same as DAR images) was generated, where the grayscale value equals the number of detected cell nuclei within the corresponding DAR pixel borders. For cells on straddle on the border of pixels, the position of the calculated cell nuclei center determined what pixel it belonged to.

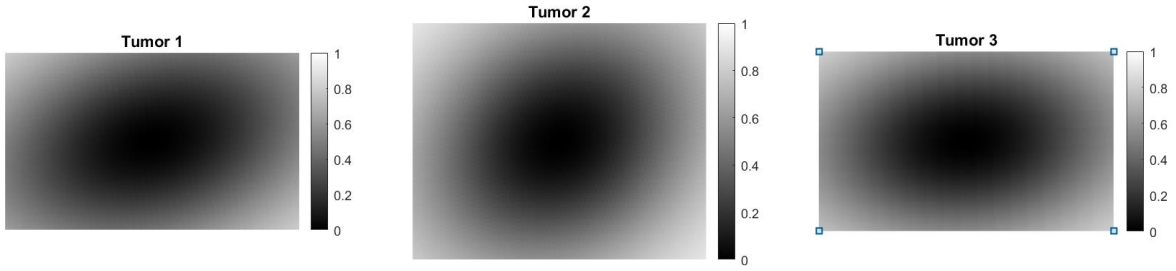
SUPPLEMENTAL TABLE 1 *Results from cell nuclei segmentation from HE-stained cryosections of LNCaP.*

	Number of pixels inside tumor borders in DAR image	Number of segmented cell nuclei	Minimum number of cell nuclei per DAR pixel	Maximum number of cell nuclei per DAR pixel
Tumor 1	18690	120482	0	29
Tumor 2	15618	58441	0	30
Tumor 3	22035	100398	0	30

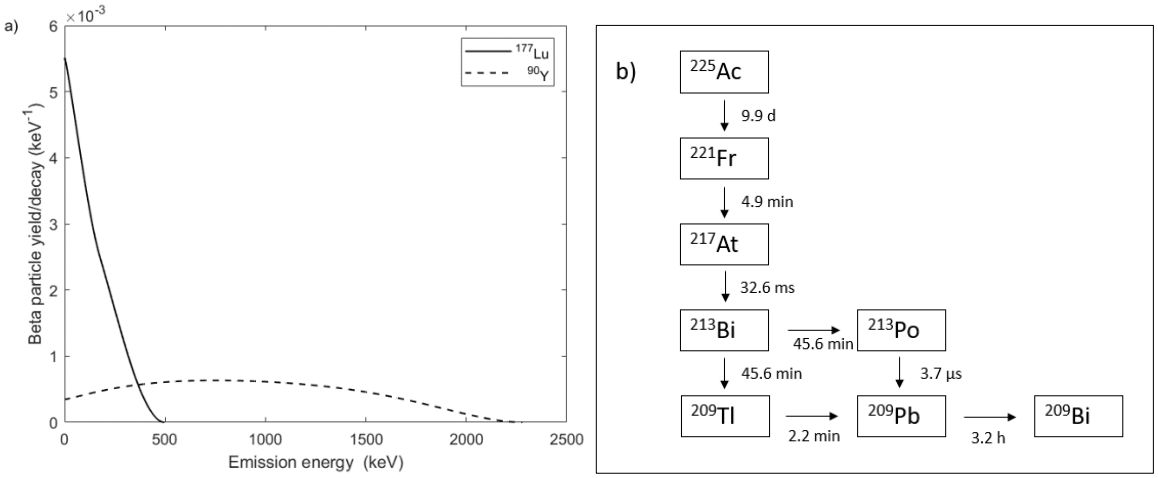
Modified DAR image

To generate a hypothetical case where the activity is primarily taken up at the edge of the xenograft due to low tumor penetration, as, e.g., can be the case in radioimmunotherapy, the DAR images were multiplied pixel-wise with an elliptical gaussian filter (SUPPLEMENTAL FIGURE 1) matched to the approximate size and shape of each tumor xenograft section. The grayscale values of these modified activity distributions were also normalized.

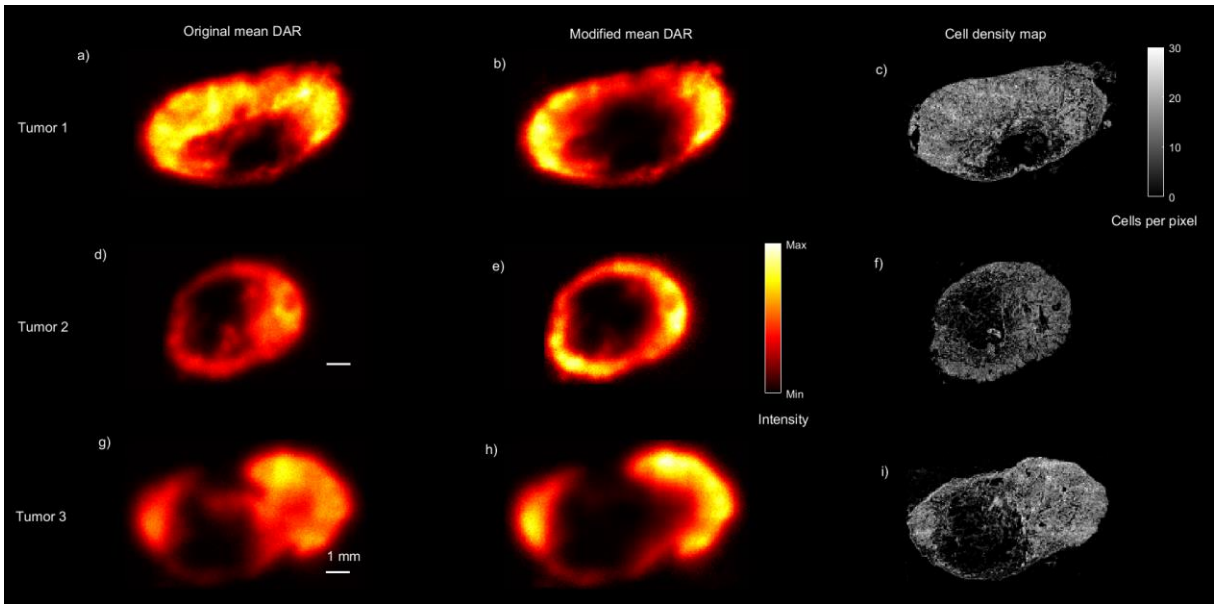
Supplemental Figures



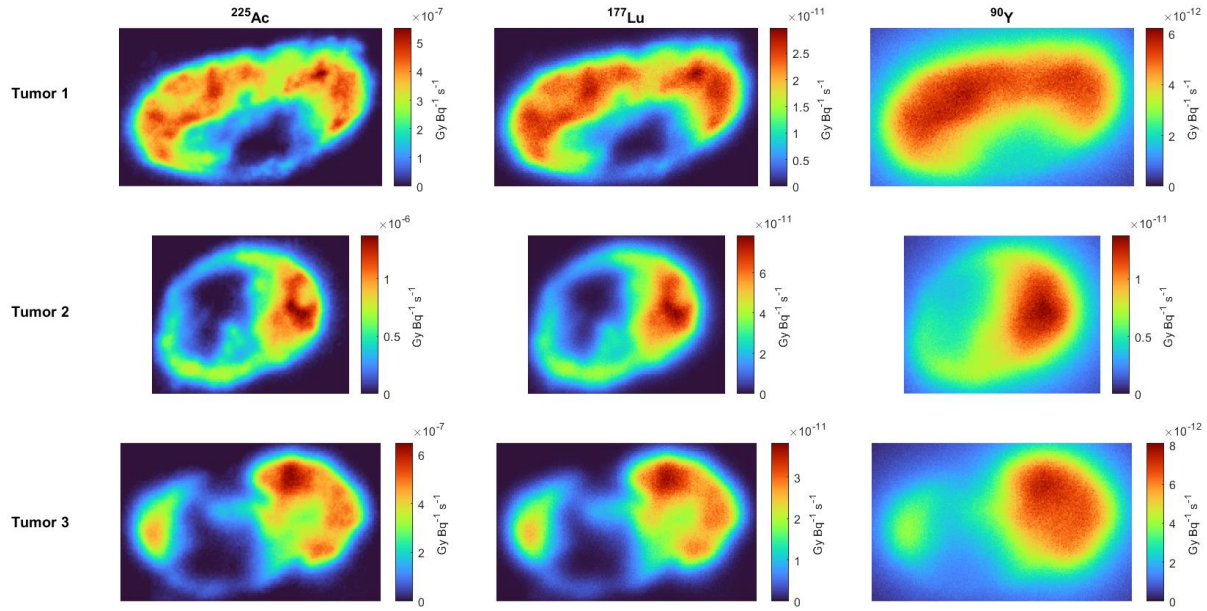
SUPPLEMENTAL FIGURE 1 *Oval gaussian filters used to multiply with the mean DAR images to generate the modified activity distributions. The grayscale values increase gradually from the center of the filter, meaning activity at the DAR image center will be greatly reduced while activity at the edges is less affected.*



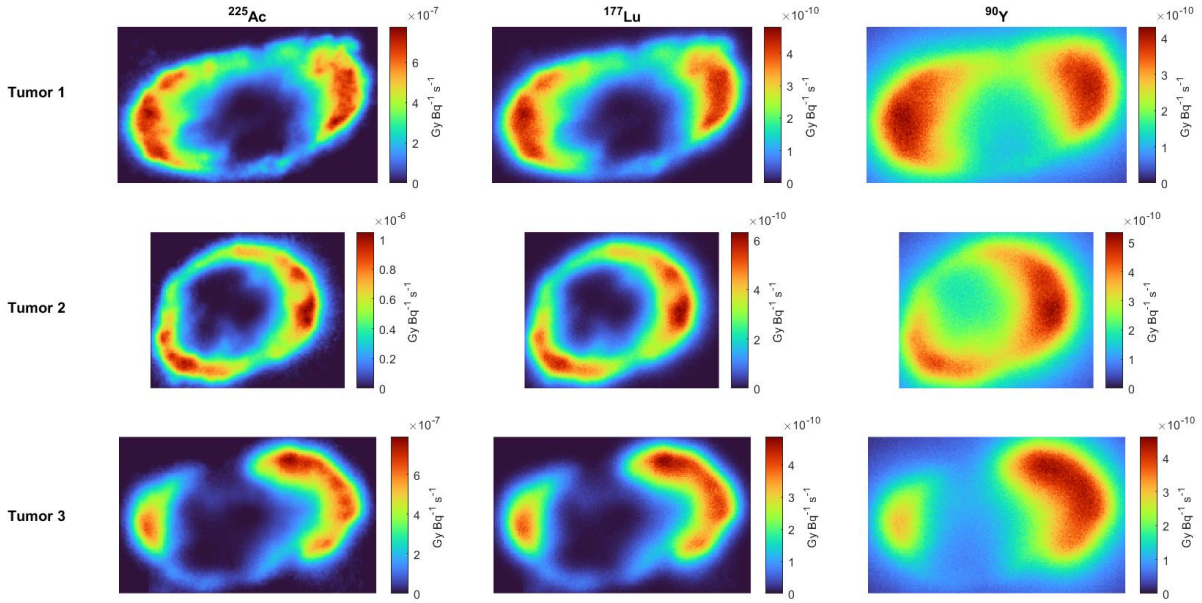
SUPPLEMENTAL FIGURE 2 *Beta energy spectrum for ^{177}Lu and ^{90}Y (a) (2,3) and ^{225}Ac decay chain (b).*



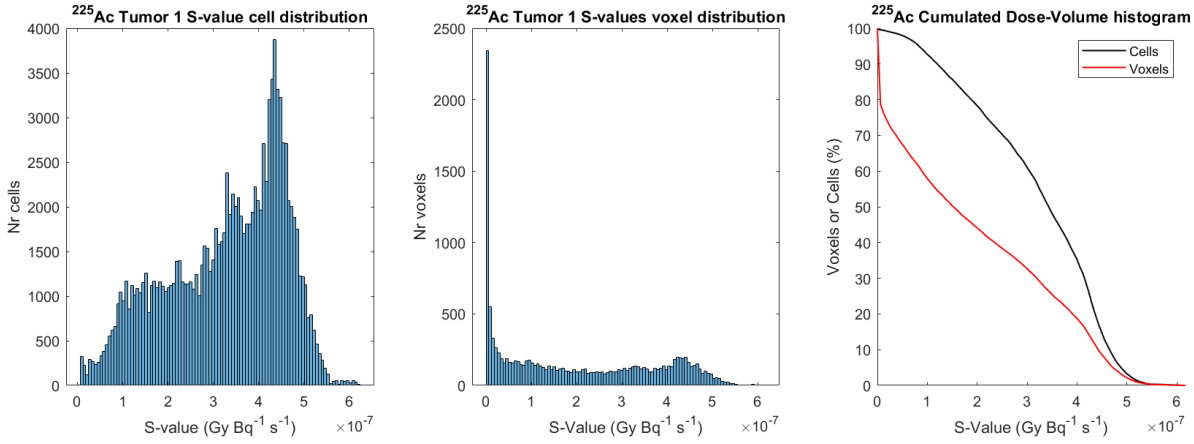
SUPPLEMENTAL FIGURE 3 *Resulting mean DAR image, modified DAR image after gauss filtering, and cell density map for tumors 1-3. The mean DAR image (a, d, and g) is the result of averaging eight sequential DAR sections after co-registration. The modified DAR images (b, e, and h) were generated by multiplying the mean DAR image with an oval gaussian filter, generating a hypothetical radioactivity distribution as if the tumor penetration was lower. The cell density map (c, f, and i) was produced from cell nucleus detection in HE-stained sections, created to relate the simulated absorbed dose in the same pixels/voxels to the number of cells, thereby generating the cell absorbed dose distribution.*



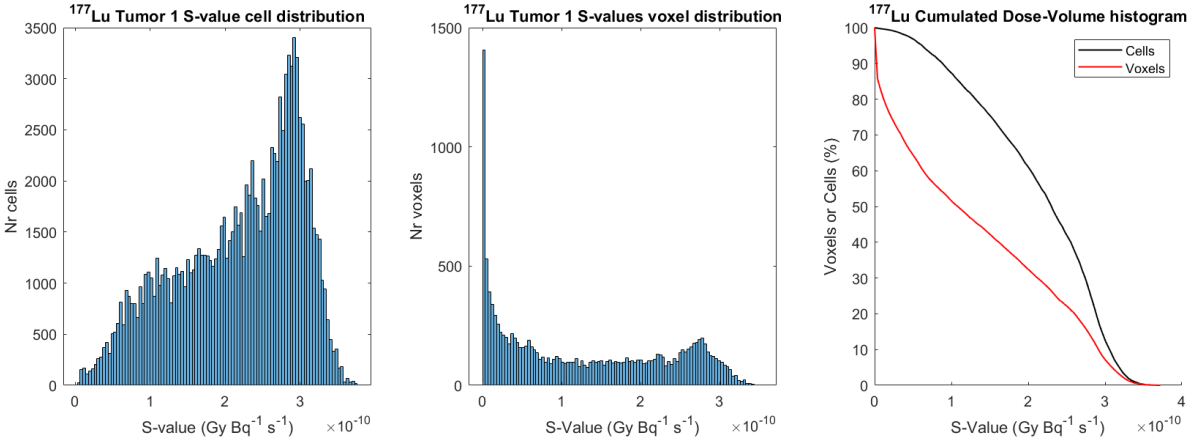
SUPPLEMENTAL FIGURE 4 *Simulated absorbed dose rate per unit activity in LNCaP tumor sections from tumor 1-3. Each pixel represents a volume of 50x50x10 µm^3. Simulations were performed for the alpha or beta emissions of ^{225}Ac (a, d, and g), ^{177}Lu (b, e, and h), and ^{90}Y (c, e, and i).*



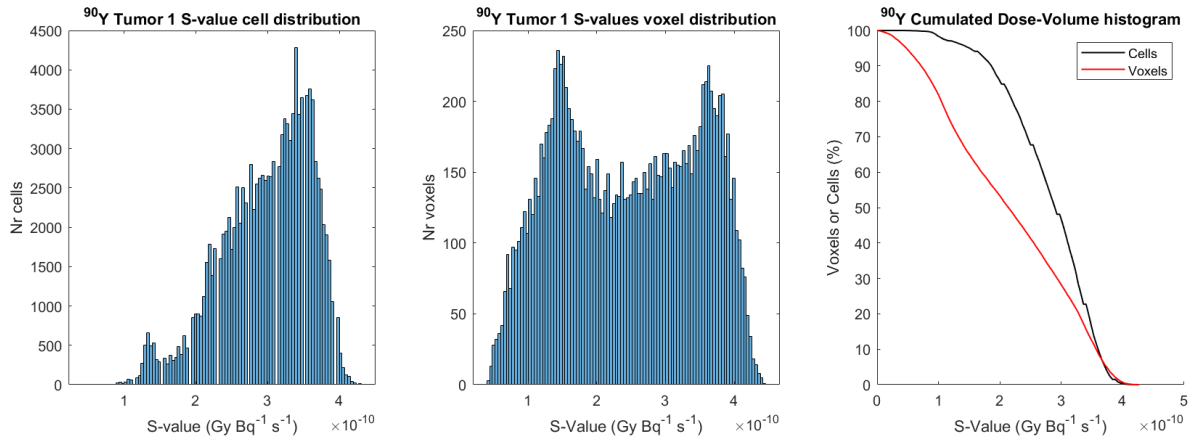
SUPPLEMENTAL FIGURE 5 *Simulated absorbed dose rate per unit activity in modified activity distributions. Simulations were performed for the alpha or beta emissions of ^{225}Ac, ^{177}Lu, and ^{90}Y.*



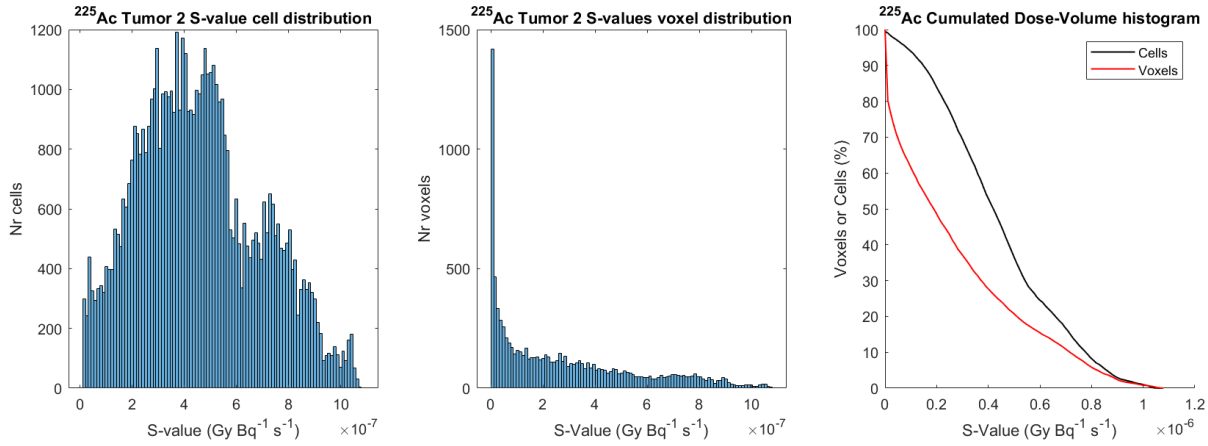
SUPPLEMENTAL FIGURE 6 *Cell s-value distribution for tumor 1 when treated with ^{225}Ac a), S-values of the pixel inside the tumor borders b), the cumulated dose-volume histogram of the S-values for either cells or pixels c).*



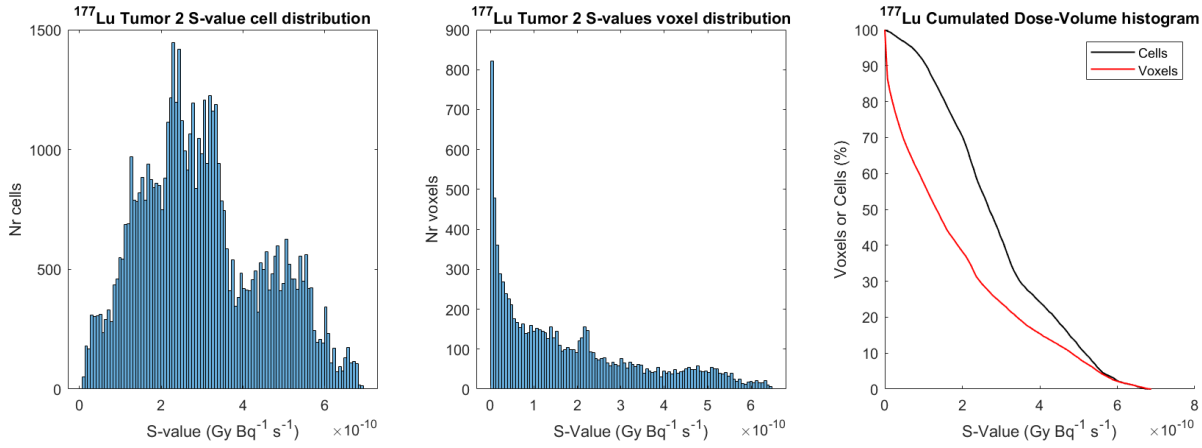
SUPPLEMENTAL FIGURE 7 *Cell s-value distribution for tumor 1 when treated with ^{177}Lu a), S-values of the pixel inside the tumor borders b), the cumulated dose-volume histogram of the S-values for either cells or pixels c).*



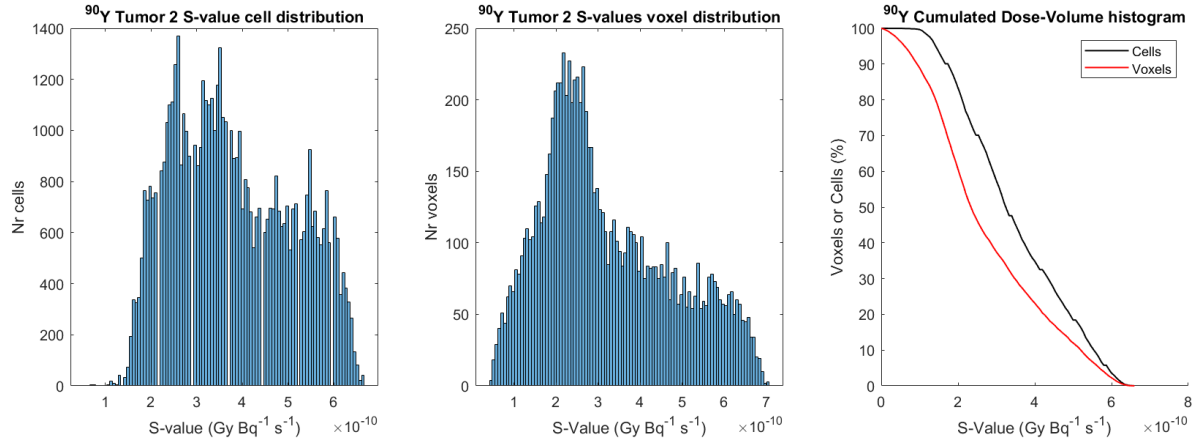
SUPPLEMENTAL FIGURE 8 *Cell s-value distribution for tumor 1 when treated with ^{90}Y a), S-values of the pixel inside the tumor borders b), the cumulated dose-volume histogram of the S-values for either cells or pixels c).*



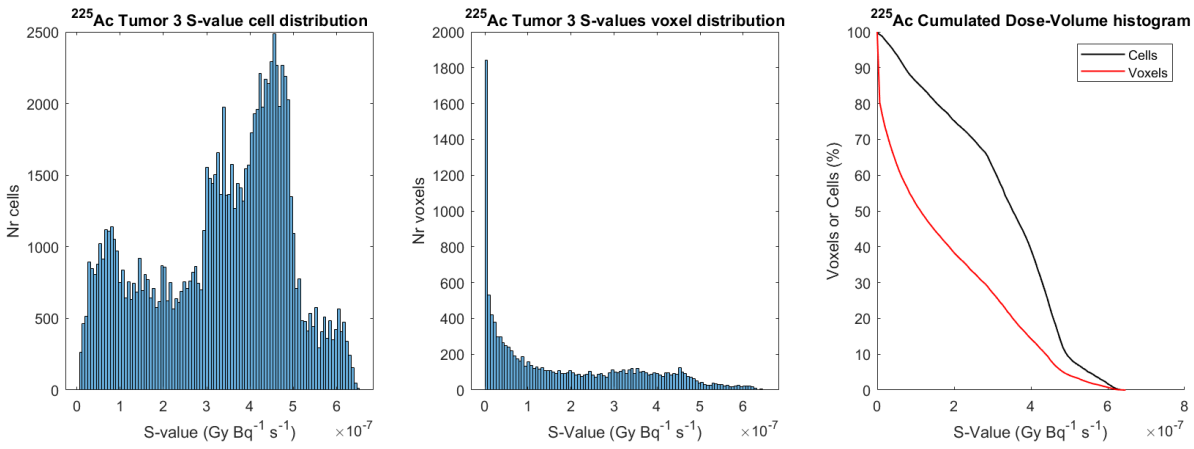
SUPPLEMENTAL FIGURE 9 *Cell s-value distribution for tumor 2 when treated with ^{225}Ac a), S-values of the pixel inside the tumor borders b), the cumulated dose-volume histogram of the S-values for either cells or pixels c).*



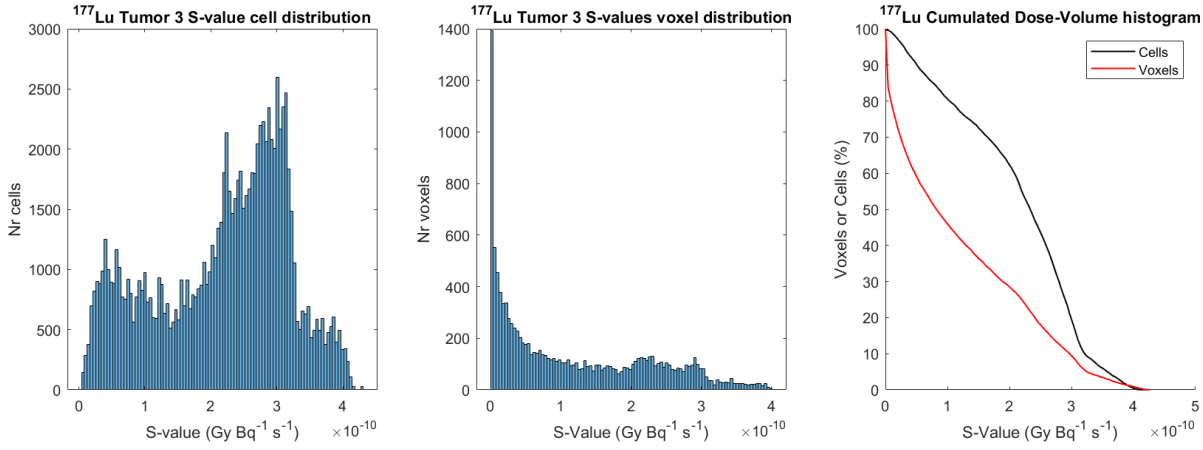
SUPPLEMENTAL FIGURE 10 *Cell s-value distribution for tumor 2 when treated with ^{177} a), S-values of the pixel inside the tumor borders b), the cumulated dose-volume histogram of the S-values for either cells or pixels c).*



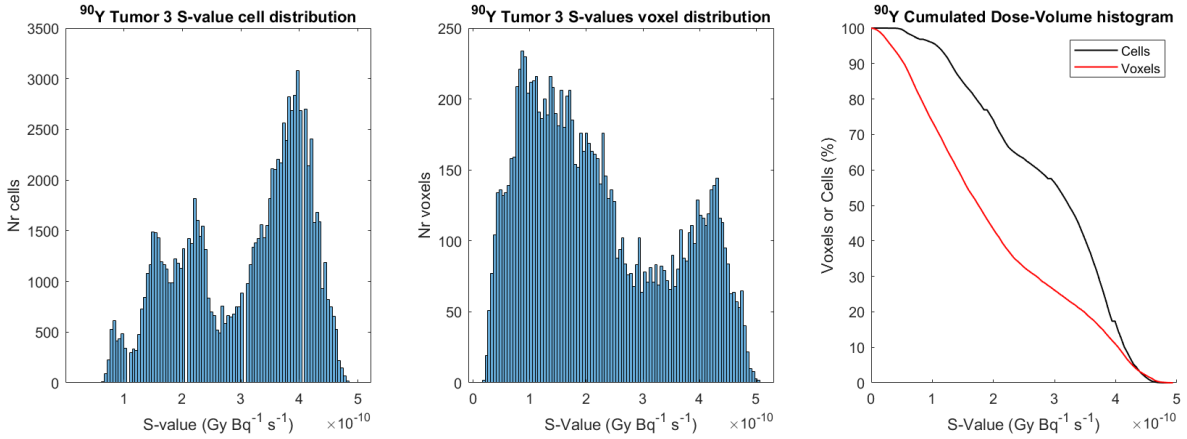
SUPPLEMENTAL FIGURE 11 *Cell s-value distribution for tumor 2 when treated with ^{90}Y a), S-values of the pixel inside the tumor borders b), the cumulated dose-volume histogram of the S-values for either cells or pixels c).*



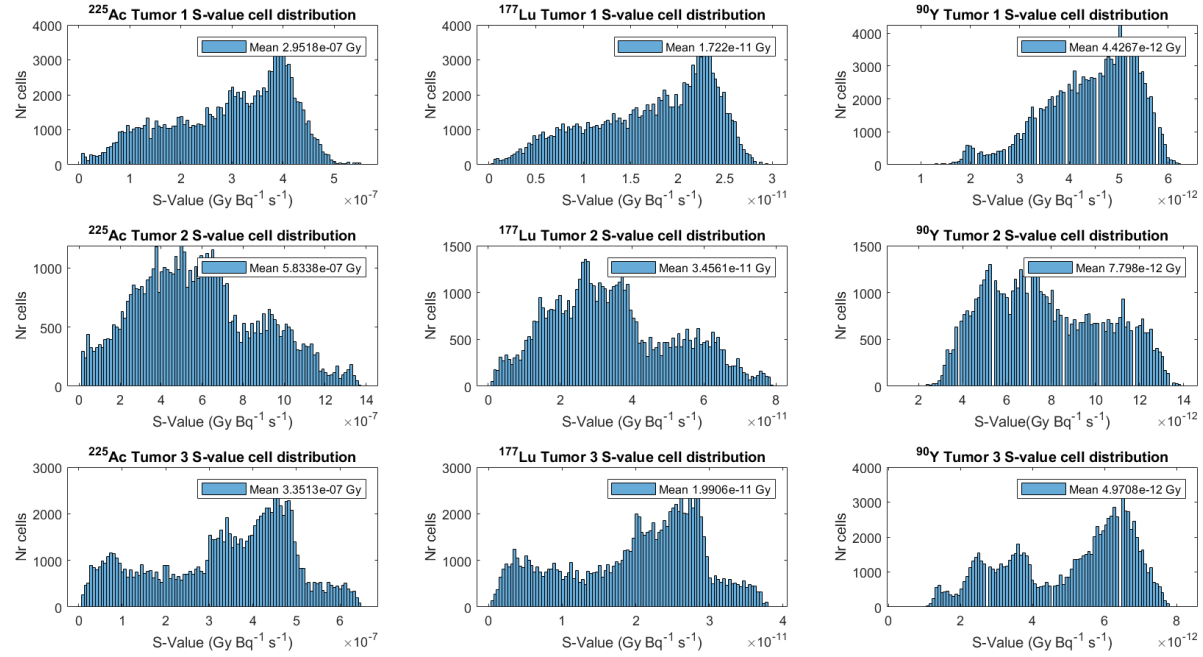
SUPPLEMENTAL FIGURE 12 *Cell s-value distribution for tumor 3 when treated with ^{225}Ac a), S-values of the pixel inside the tumor borders b), the cumulated dose-volume histogram of the S-values for either cells or pixels c).*



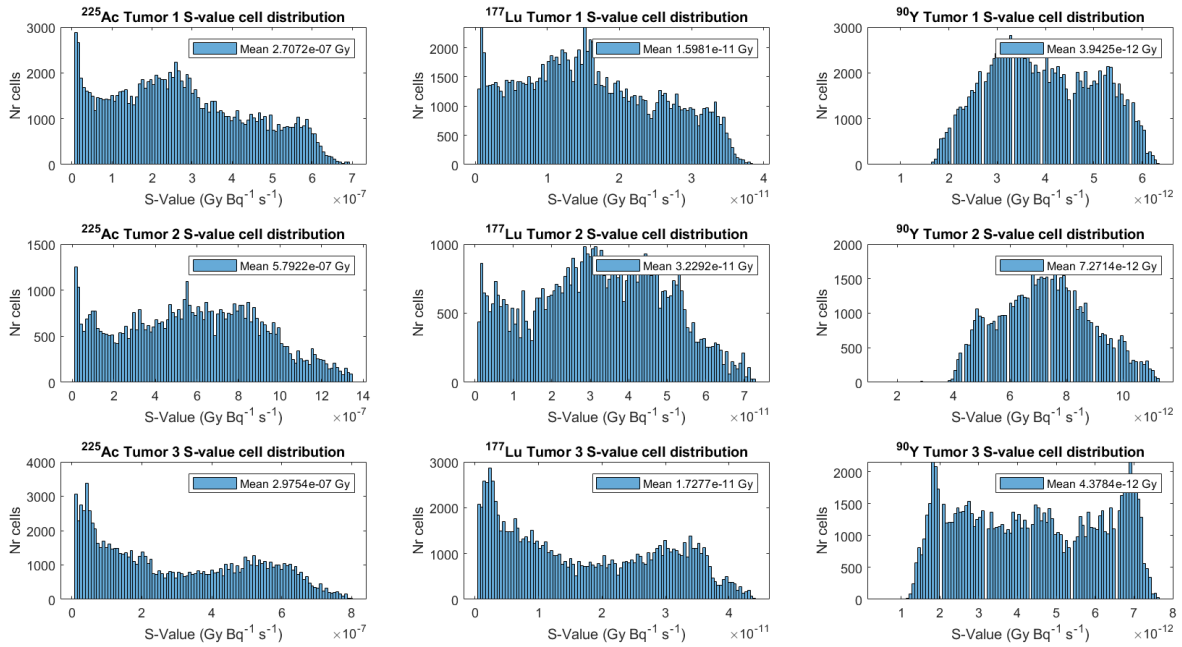
SUPPLEMENTAL FIGURE 13 *Cell s-value distribution for tumor 3 when treated with ^{177} a), S-values of the pixel inside the tumor borders b), the cumulated dose-volume histogram of the S-values for either cells or pixels c).*



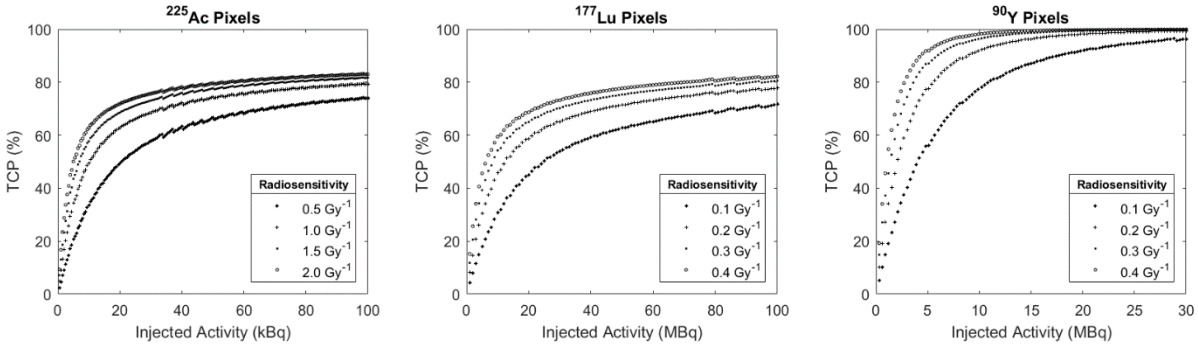
SUPPLEMENTAL FIGURE 14 *Cell s-value distribution for tumor 3 when treated with ^{90}Y a), S-values of the pixel inside the tumor borders b), the cumulated dose-volume histogram of the S-values for either cells or pixels c).*



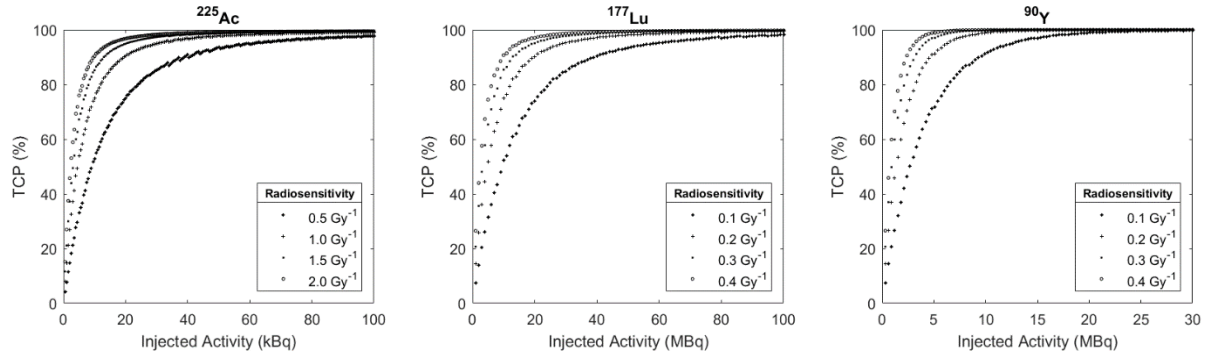
SUPPLEMENTAL FIGURE 15 *Simulated cell S-value distributions for ^{225}Ac, ^{177}Lu, and ^{90}Y in Tumor 1, 2, and 3. The absorbed dose per decay distribution for each tumor and specific radionuclide is calculated by dividing the absorbed dose image from the GATE DoseActor by the primary simulated events, i.e., dividing the absorbed dose by the total number of decays.*



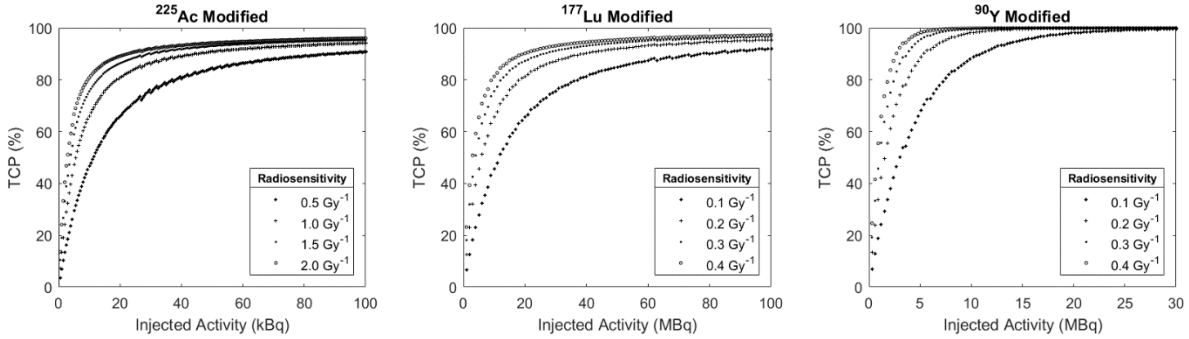
SUPPLEMENTAL FIGURE 16 *Simulated cell S-value distributions in the modified activity distribution for ^{225}Ac, ^{177}Lu, and ^{90}Y in tumors 1, 2, and 3.*



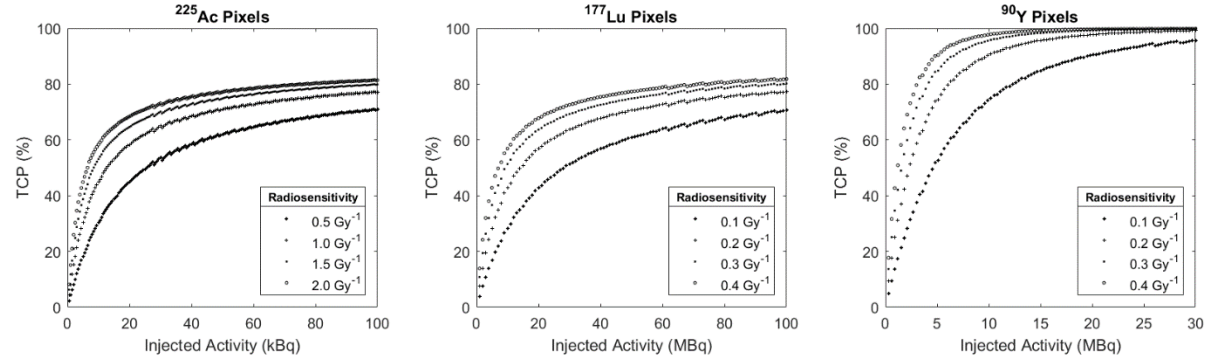
SUPPLEMENTAL FIGURE 17 *TCP vs. injected activity in tumor 1 when considering pixels. ^{225}Ac in a), ^{177}Lu in b), and ^{90}Y in c).*



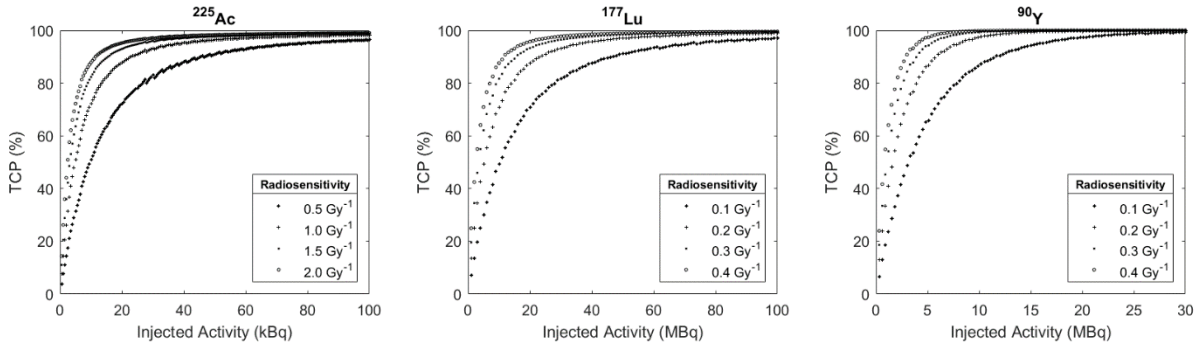
SUPPLEMENTAL FIGURE 18 *TCP vs. injected activity in tumor 1 when considering cells. ^{225}Ac in a), ^{177}Lu in b), and ^{90}Y in c).*



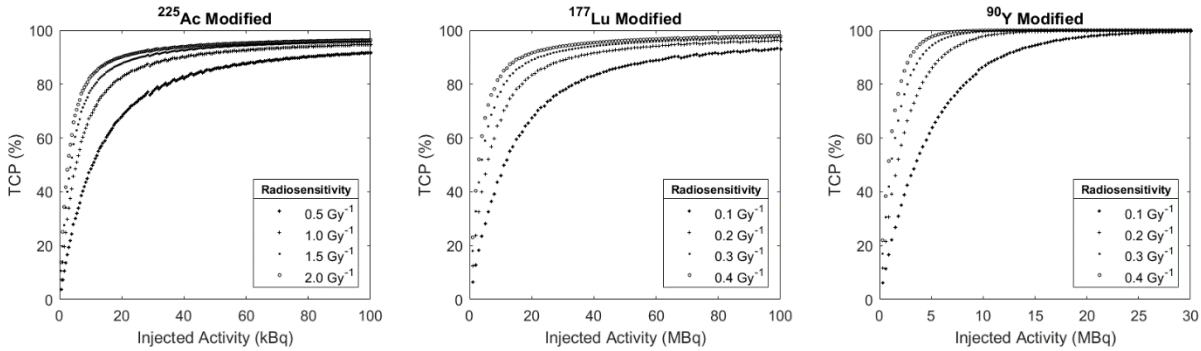
SUPPLEMENTAL FIGURE 19 *TCP vs. injected activity in tumor 1 when considering cells for the modified activity distribution. ^{225}Ac in a), ^{177}Lu in b), and ^{90}Y in c).*



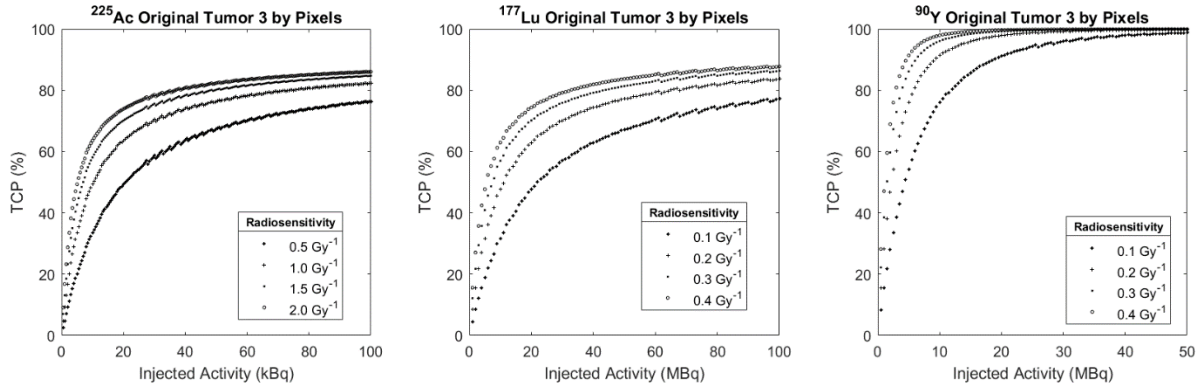
SUPPLEMENTAL FIGURE 20 *TCP vs. injected activity in tumor 2 when considering pixels. ^{225}Ac in a), ^{177}Lu in b), and ^{90}Y in c).*



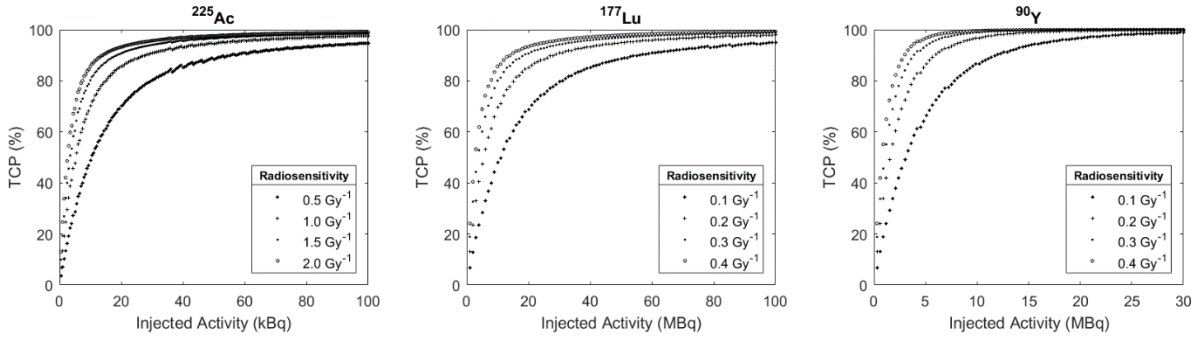
SUPPLEMENTAL FIGURE 21 *TCP vs. injected activity in tumor 2 when considering cells. ^{225}Ac in a), ^{177}Lu in b), and ^{90}Y in c).*



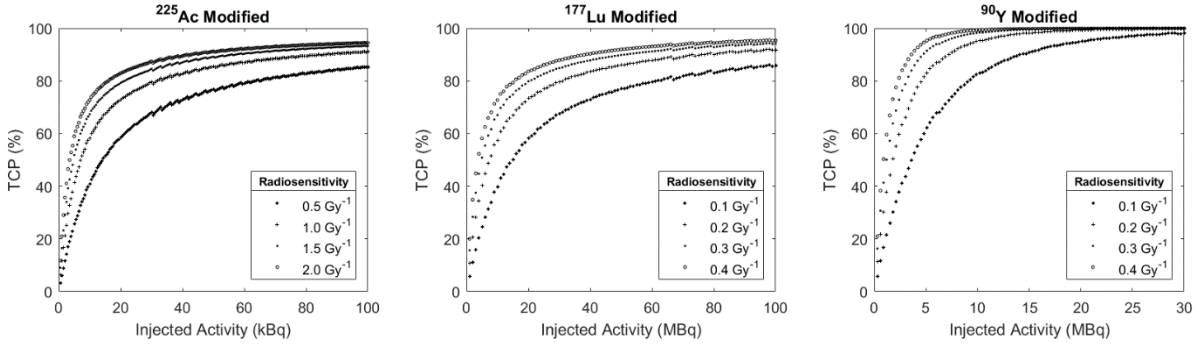
SUPPLEMENTAL FIGURE 22 *TCP vs. injected activity in tumor 2 when considering cells for the modified activity distribution. ^{225}Ac in a), ^{177}Lu in b), and ^{90}Y in c).*



SUPPLEMENTAL FIGURE 23 *TCP vs. injected activity in tumor 3 when considering pixels. ^{225}Ac in a), ^{177}Lu in b), and ^{90}Y in c).*



SUPPLEMENTAL FIGURE 24 *TCP vs. injected activity in tumor 3 when considering cells. ^{225}Ac in a), ^{177}Lu in b), and ^{90}Y in c).*



SUPPLEMENTAL FIGURE 25 *TCP vs. injected activity in tumor 3 when considering cells for the modified activity distribution. ^{225}Ac in a), ^{177}Lu in b), and ^{90}Y in c).*

Supplemental References

1. Kristiansson A, Orbom A, Ahlstedt J, et al. (177)Lu-PSMA-617 Therapy in Mice, with or without the Antioxidant alpha(1)-Microglobulin (A1M), Including Kidney Damage Assessment Using (99m)Tc-MAG3 Imaging. *Biomolecules.* 2021;11(2):263.

2. Mougeot X. Towards high-precision calculation of electron capture decays. *Appl Radiat Isot.* 2019;154:108884.

3. Mougeot X. Erratum: Reliability of usual assumptions in the calculation of beta and neurino spectra [Phys. Rev. C 91, 055504 (2015)]. *Physical Review C.* 2015;92:059902.