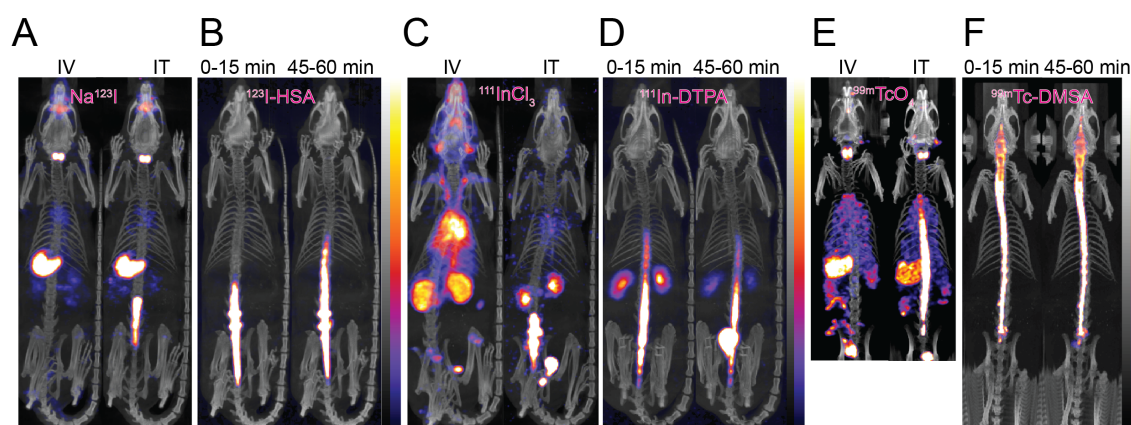
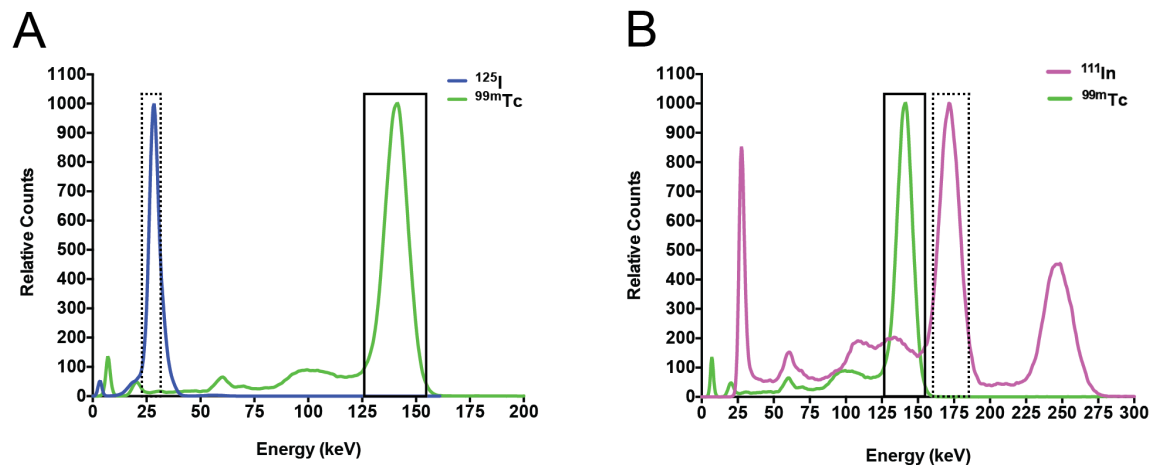
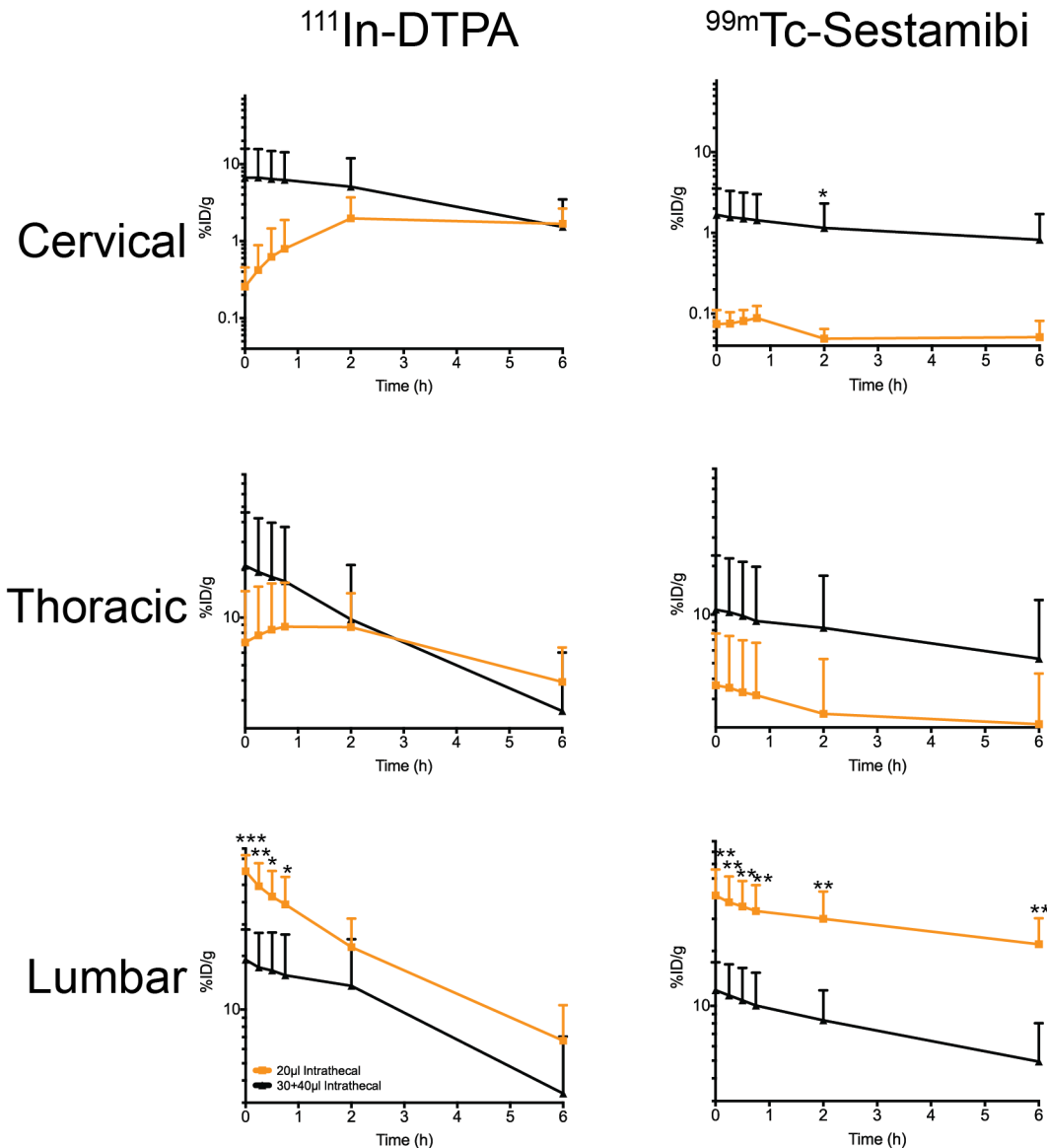




Supplementary Figure S1: High-resolution SPECT-CT scans comparing the distribution of free radionuclides to that of drug surrogate molecules radiolabeled with radionuclides after either intravenous or intrathecal injection. **(A, C, E)** High-resolution SPECT-CT images 0-15 minutes post-intravenous (IV) or –intrathecal (IT) injection of Na^{123}I , $^{111}\text{InCl}_3$, and $^{99\text{m}}\text{TcO}_4$ respectively ($n=1$ experiment conducted for each compound and route of administration). Following intrathecal injection, all three agents displayed rapid clearance into peripheral organs. Na^{123}I and $^{99\text{m}}\text{TcO}_4$ exhibited a characteristic pattern of uptake into the stomach, thyroid, and nasal epithelium, while $^{111}\text{InCl}_3$ was cleared renally. (IV injections were all delivered via a 100 μl bolus, Intrathecal injections: 10 μl bolus for Na^{123}I and $^{111}\text{InCl}_3$, 30 μl + 40 μl saline flush bolus through a catheter for $^{99\text{m}}\text{TcO}_4$). **(B)** After covalent attachment of ^{123}I to HSA protein, SPECT-CT scans 0-15 minutes and 45-60 minutes after lumbar injection showed that the tracer was highly retained within the IT space (representative data shown from one animal of a cohort of $n=4$). **(D,F)** Conversely, the small molecule ^{111}In -DTPA (M.W. 545 Da) **(D)** displayed renal clearance from the spinal IT space within the same time frame (representative data shown from one animal of a cohort of $n=4$) while the similarly sized $^{99\text{m}}\text{Tc}$ -DMSA (M.W. 492 Da) **(F)** did not ($n=1$ experiment conducted).

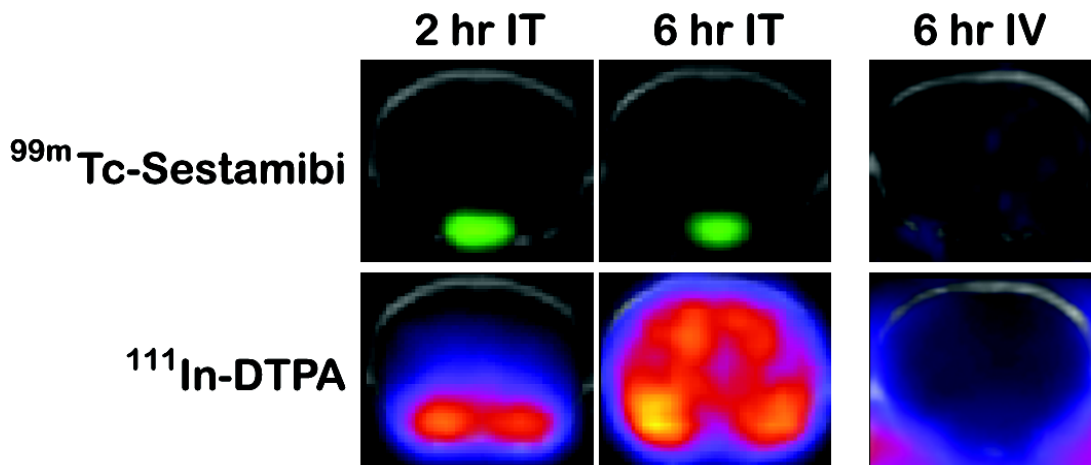


Supplementary Figure S2: Gamma ray emission energy spectra from the radioisotopes used in dual label SPECT imaging studies. The boxes highlight the major peaks used for analysis. See methods for further details.



Supplementary Figure S3: Kinetics of ^{111}In -DTPA and $^{99\text{m}}\text{Tc}$ -sestamibi movement through the CSF at different levels of the spinal column as measured by dynamic dual SPECT-CT imaging and plotted as time-activity curves (from data presented in Figure 4, F and I). Data points are plotted on a logarithmic scale and represent the mean $\pm$ S.D. * $P < 0.05$, ** $P < 0.01$ by unpaired two-tailed Student's t -test. 2-way repeated measures ANOVA analyses revealed significant differences in overall

uptake of ^{111}In -DTPA (** $P<0.01$) and $^{99\text{m}}\text{Tc}$ -Sestamibi (** $P<0.01$) into the lumbar ROI over the time-course of the experiment attributable to the difference in volume of bolus administered between the two groups. Mean AUC Values for ^{111}In -DTPA: (lumbar: 20 μl IT= 132, 30+40 μl IT= 64.9), (thoracic: 20 μl IT= 42.1, 30+40 μl IT= 55.4), (cervical: 20 μl IT= 9.4, 30+40 μl IT= 25.2). Mean AUC Values for $^{99\text{m}}\text{Tc}$ -sestamibi: (lumbar: 20 μl IT= 170, 30+40 μl IT= 46.3), (thoracic: 20 μl IT= 15.1, 30+40 μl IT= 45.6), (cervical: 20 μl IT= 0.3, 30+40 μl IT= 6.7).



Supplementary Figure S4: Comparison of brain parenchymal penetration by intrathecal vs. intravenous ^{99m}Tc -Sestamibi vs. ^{111}In -DTPA. Progressive brain parenchymal accumulation of ^{99m}Tc -Sestamibi and ^{111}In -DTPA is shown following their IT (intracisternal) co-injection at 2 and 6 hrs as taken from Figure 6D. For comparison, the right panels show typical 6 hr results from similar amount of these agents given IV. Note that lack of brain signal following IV dosing. IV results are exemplary of experiments performed at least 4 times for ^{99m}Tc -Sestamibi and twice for ^{111}In -DTPA.

Supplementary Video 1: 3D reconstruction of lumbar-administered India ink highlighting the rat CSF-containing intrathecal space.

Supplementary Video 2: Fly-through movie of a 3D reconstruction (axial view) made from serial sagittally-sliced block-face cryosections of a rat following lumbar injection of Evans blue dye.

Supplementary Video 3: Time-lapsed planar scintigraphy scans from a rat following catheterized lumbar injection of a mixed solution of ^{125}I -HSA (top) and $^{99m}\text{TcO}_4$ (bottom). The synchronized movies are from the same animal with the signals detected using separate energy windows.

Supplementary Video 4: Time-lapsed planar scintigraphy scans from a rat following catheterized lumbar injection of a mixed solution of ^{99m}Tc -DMSA (top

left) and ^{111}In -DTPA (bottom left). The synchronized movies are from the same animal with the signals detected using separate energy windows.

Supplementary Video 5: Maximum intensity projection images from rats following lumbar injection of a mixed solution of ^{111}In -DTPA (fire scale) and $^{99\text{m}}\text{Tc}$ -sestamibi (green scale). Top row = 20 μl dose volume, bottom row = 30 μl + 40 μl saline flush.