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☐ Initial submission ☐ Revised version ☒ Final submission

Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

Experimental design

1. Sample size

Describe how sample size was determined.

Sample size calculations were not determined prior to experiment, however all well plates were conducted with triplicate wells for reproducibility. controls also in triplicate were done for particles alone, and radionuclide alone. Replicate experiments were also conducted and agreed with initial results when corrected for radionuclide decay. Radionuclide only radiance values were done based upon >5 data points each with 4 replicates, and normalized to radiance per unit of activity. SPECT studies were also done in triplicate, though Figure 4a and b were qualitatively done as single experiments with a HPGe detector to confirm presence of new X-Rays. Note only one sample could be measured at a time on the HPGe detector. In vivo experiments were sparing, testing a maximum of n=3 mice to highlight imaging capability by optical and SPECT methodologies. Tumor bearing mice in 5a-d are single mice injected with either nanoparticle or radiotherapeutic and nanoparticle.

2. Data exclusions

Describe any data exclusions.

Data sets excluded were done so based upon poor mixing and high SD of the triplicate wells. nanoparticles that were high light absorbers (like Ge) were removed from main figures to simplify results. SPECT scans excluded were done based upon image acquisition and processing faults (wrong energy calibration or wrong energy map).

3. Replication

Describe whether the experimental findings were reliably reproduced.

Well plate experiments were done in triplicate and have been repeated at least two times. preparation of nanoparticle solutions was standardized to a concentration (1e12/mL) and made fresh before measurements.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

Well plate studies were not randomized, but controlled where each plate image acquired contained the nanoparticles without any radionuclide, the radionuclide alone, and the select nanoparticles with the radionuclide. Radiances of controls were then compared across plates to check for bias (such as overestimation of radiance based upon bleed over from adjacent wells). Radioluminesence studies were additionally done to confirm effect of radionuclide decay from adjacent wells exciting other wells. Animals used for in vivo imaging were randomized for selection in imaging experiments.

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

No formal blinding was done for these experiments

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ A statement indicating how many times each experiment was replicated
☐	☒ The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
☐	☒ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒ The test results (e.g. *P* values) given as exact values whenever possible and with confidence intervals noted
☐	☒ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒ Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.

All IVIS ROIs were drawn in Living Image V4.5 and tabulated in Microsoft Excel and the mean and standard deviation plotted in Graphpad Prism V6 and V7. SPECT data was processed on a workstation running invivoscope and HISPEC. ROIs for SPECT images were drawn from DICOM images using Amide V 1.0.4. Only code written for this manuscript encompasses an addendum to the radionuclide tables in the MEDISO nanoSPECT to include X-rays energies based upon the element NP used.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

Nanoparticles used in this manuscript can be acquired commercially. only restriction on materials would be the end user's access to radionuclides.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

No antibodies or proteins were used in this manuscript

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

Figure 5a-d used the BT-474 cell line which was implanted orthotopically under the mammary pad of female nude mice. BT-474 cell lines were acquired from ATCC and frozen stocks authenticated to confirm adherence to STR profile.

b. Describe the method of cell line authentication used.

Cell lines were not authenticated for the BT-474 cell line.

c. Report whether the cell lines were tested for mycoplasma contamination.

Cells were tested for mycoplasm contamination and determined to be negative.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

No commonly misidentified cell lines were used

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.	Athymic nude female mice were used for imaging studies (phantoms as in fig 3) and for tumor bearing mice in fig 5a-d. Athymic nude male mice without tumors were used in fig 5e-g.

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.	Manuscript did not involve the use of human research participants