Supplementary Information (SI) for Nanoscale.

Supporting Information

Liposome Biodistribution Mapping with in vivo X-ray Fluorescence Imaging

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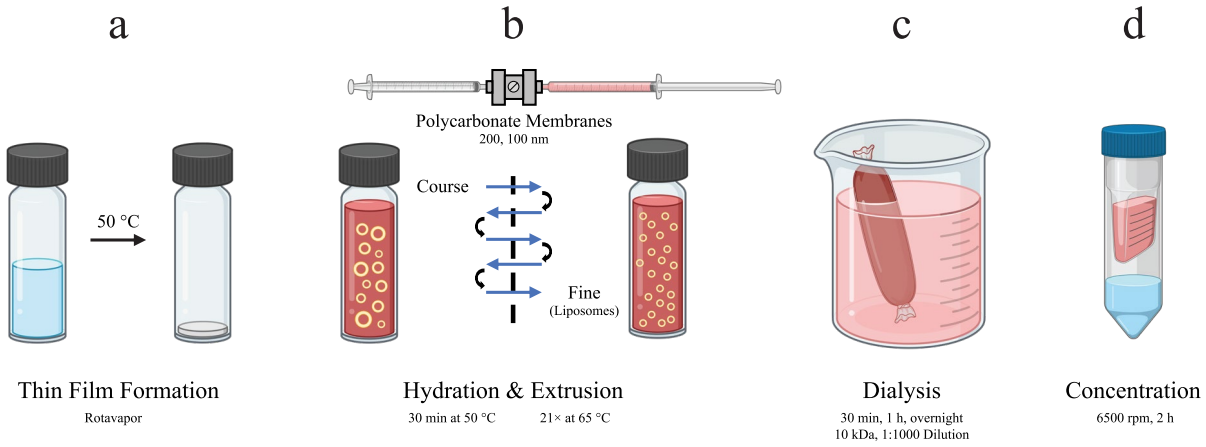

Fig. S1.

Synthesis Scheme. Ruthenium-encapsulated liposomes (Ru-Lipo) were synthesized with a thin-film hydration method. (**a**) A solution of lipids in chloroform was evaporated using a rotavapor at 50 °C, forming a thin film. (**b**) The film was hydrated with an aqueous solution of $Ru(bpy)_3$, followed by subsequent extrusions with polycarbonate membrane, leading to the formation of uniform Ru-Lipo. (**c**) The sample was dialyzed multiple times and (**d**) concentrated with centrifuge filter units.

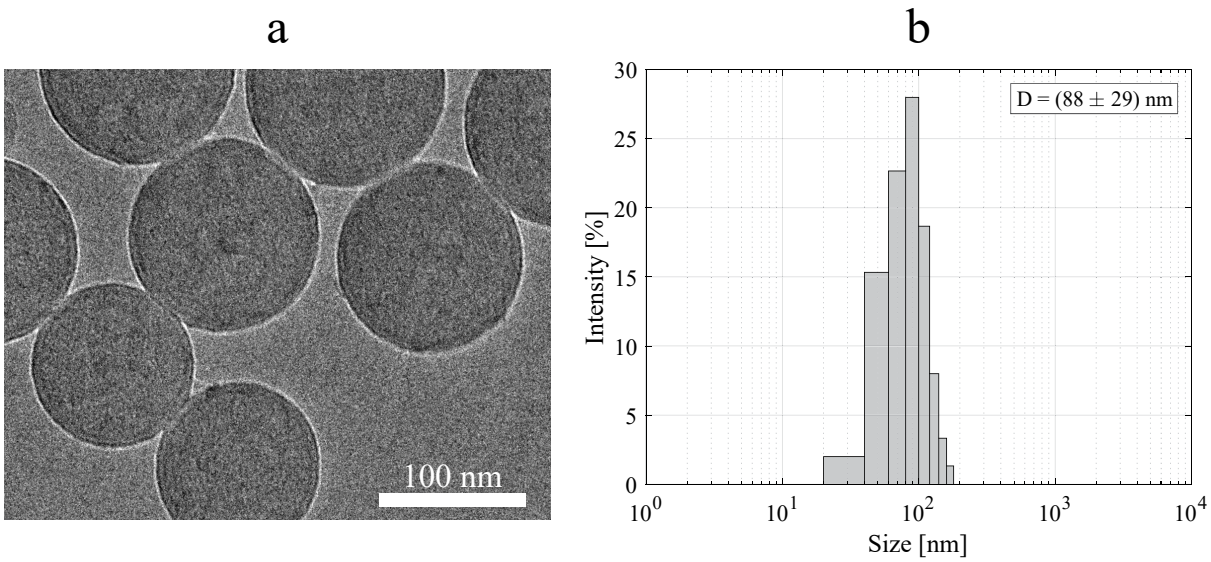

Fig. S2.

Morphological characterization. (**a**) High-resolution Cryo-TEM micrograph of ruthenium-encapsulated liposomes, Ru-Lipo, showing the lipid bilayer structure. Scale bar, 100 nm. (**b**) Size distribution histogram of Ru-Lipo obtained from the Cryo-TEM micrographs, leading to an estimated diameter of 88 ± 29 nm.

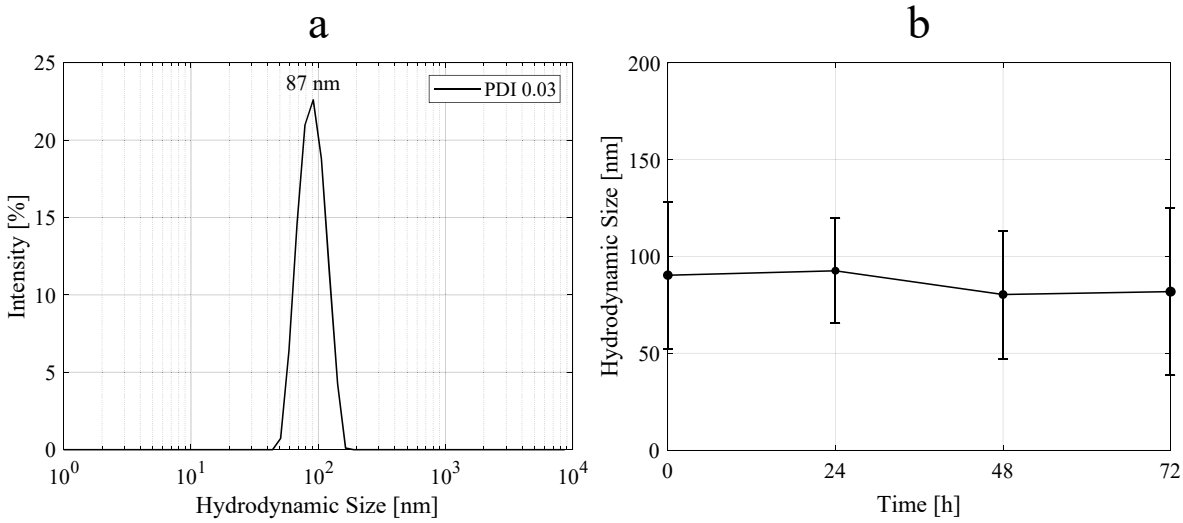

Fig. S3.

Stability characterization. (**a**) Hydrodynamic size distribution of the ruthenium-encapsulated liposomes, Ru-Lipo and (**b**) longitudinal study in saline solution (0.9 %) at 37 °C (± SD).

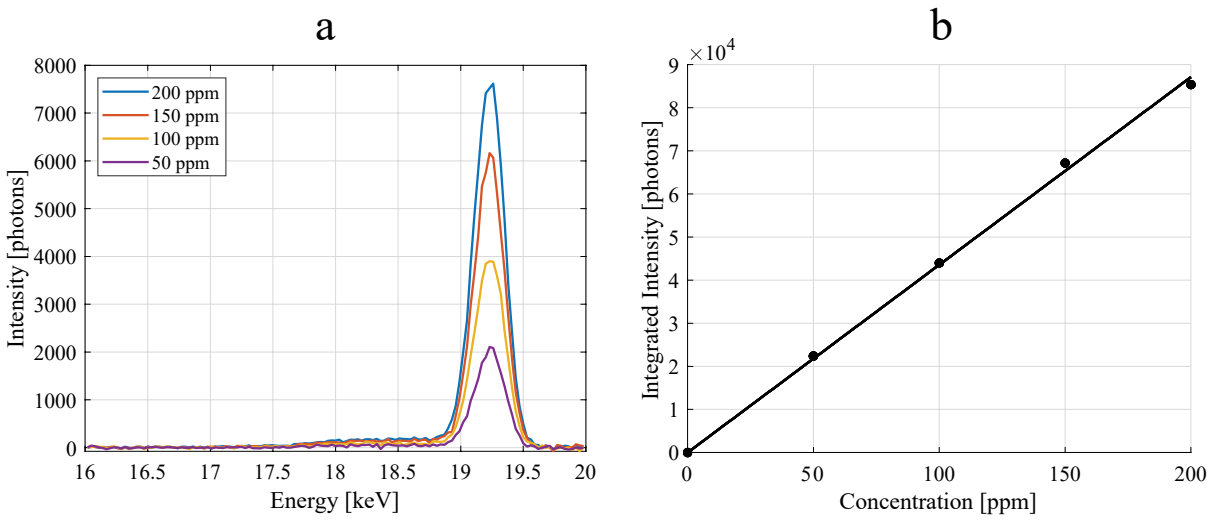

Fig. S4.

X-ray fluorescence (XRF) properties of Ru-Lipo. (**a**) XRF Kα emission peaks as a function of Ru-Lipo's concentration, after background removal. (**b**) Calibration curve for XRF intensity as a function of the concentration of Ru standards.

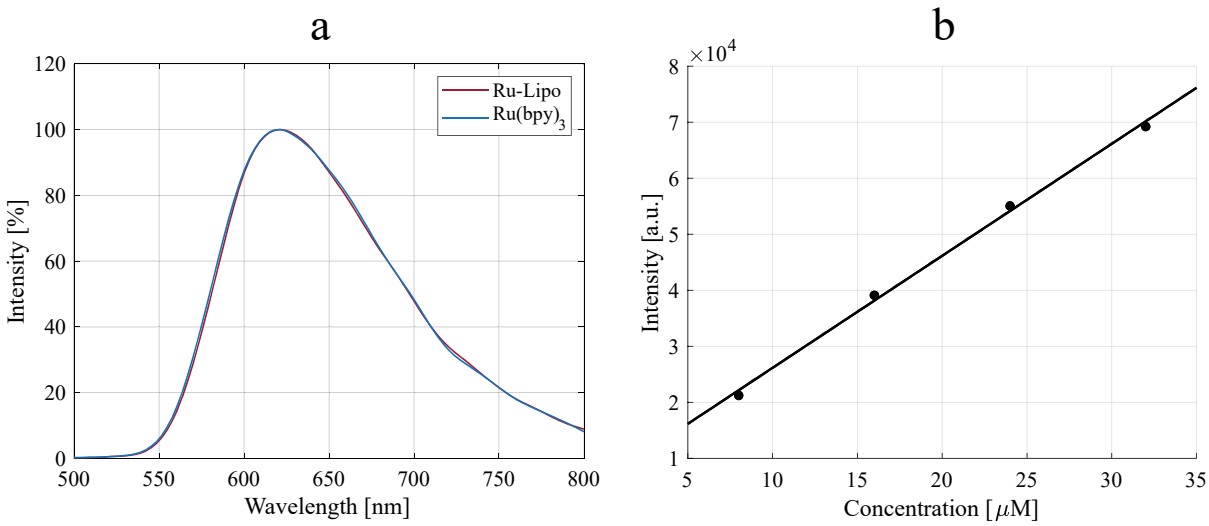

Fig. S5.

Optical fluorescence properties of Ru-Lipo. (**a**) Fluorescence emission spectra of Ru-Lipo (in red) and the free dye, $Ru(bpy)_3$ (in blue). (**b**) Calibration curve for optical fluorescence intensity as a function of the concentration of $Ru(bpy)_3$.

Table S1. Ruthenium-encapsulated liposome (Ru-Lipo) properties. Estimated values for encapsulation efficiency (EE), Ru-to-Lipid ratio, Load-to-Lipid ratio, initial and effective lipid ratios (w/w).

Encapsulation Efficiency (EE)	Ru-to-Lipid Ratio (w/w)	Load-to-Lipid Ratio (w/w)	Initial Lipid Ratio (DSPC:CH:DSPE-PEG)	Effective Lipid Ratio (DSPC:CH:DSPE-PEG)
10.00 %	1.80 %	13.2 %	3:1:1	3:0.8:0.7

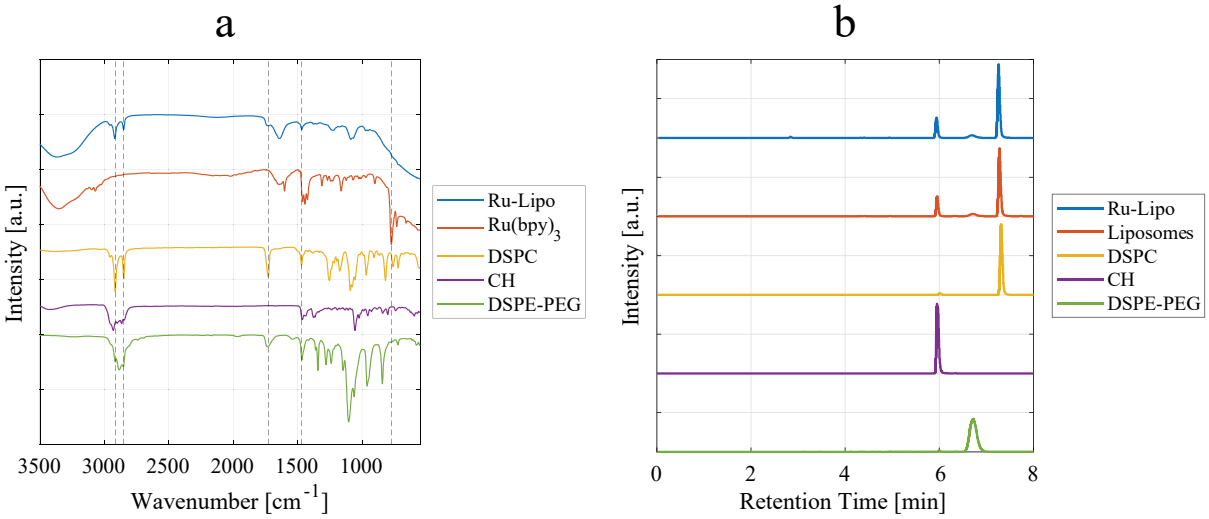

Fig. S6.

Structural characterization. (**a**) FT-IR spectra of Ru-Lipo (blue), $Ru(bpy)_3$ (orange), DSPC (yellow), CH (purple), and DSPE-PEG (green). (**b**) UPLC plots of diluted samples containing Ru-Lipo (blue), empty liposomes (orange), DSPC (yellow), CH (purple), and DSPE-PEG (green).

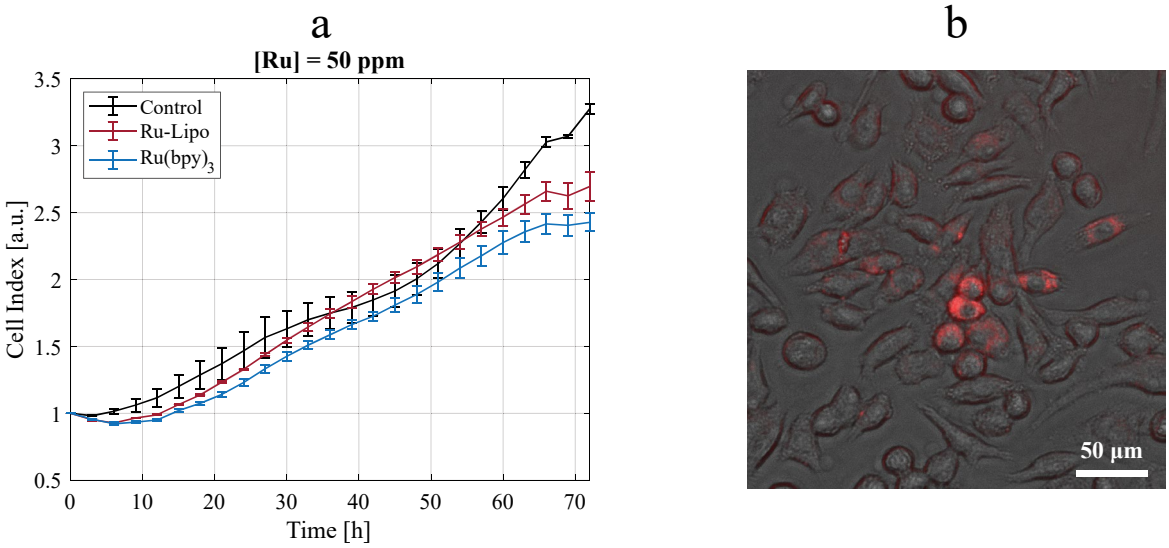

Fig. S7.

Cell studies. (**a**) Real-time cell analysis (RTCA) assay on macrophages (RAW 264.7), after exposure to ruthenium-encapsulated liposomes (Ru-Lipo, in red) and free ruthenium dye, $Ru(bpy)_3$ (in blue) with a ruthenium concentration of 50 ppm. The cell index values are compared to unexposed (negative) control cells (black). Measurements were made in triplicates (± SD). (**b**) Live images of RAW 264.7 macrophages incubated with Ru-Lipo ([Ru] = 50 ppm) for 1 h. Optical fluorescence signal from Ru-Lipo is shown in red. Trans-luminescence signal is included to highlight cell morphology. Scale bar, 50 µm.

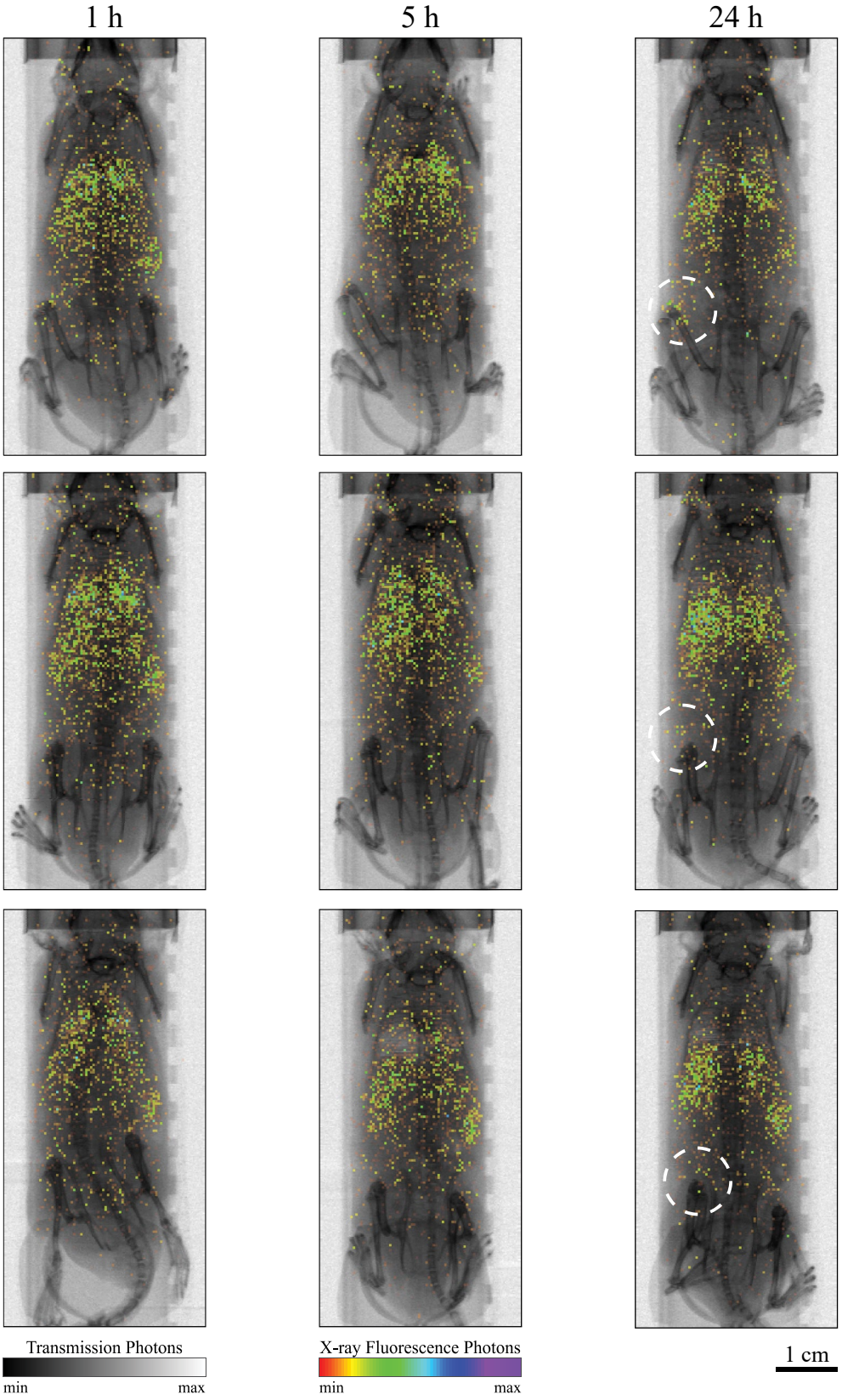

Fig. S8.

In Vivo X-ray Fluorescence Imaging. Mice injected with Ru-Lipo were imaged after 1 h, 5 h, and 24 h. X-ray fluorescence signal overlaid on transmitted photons. Tumor area is indicated with a dashed white circle. Scale bar, 1 cm.

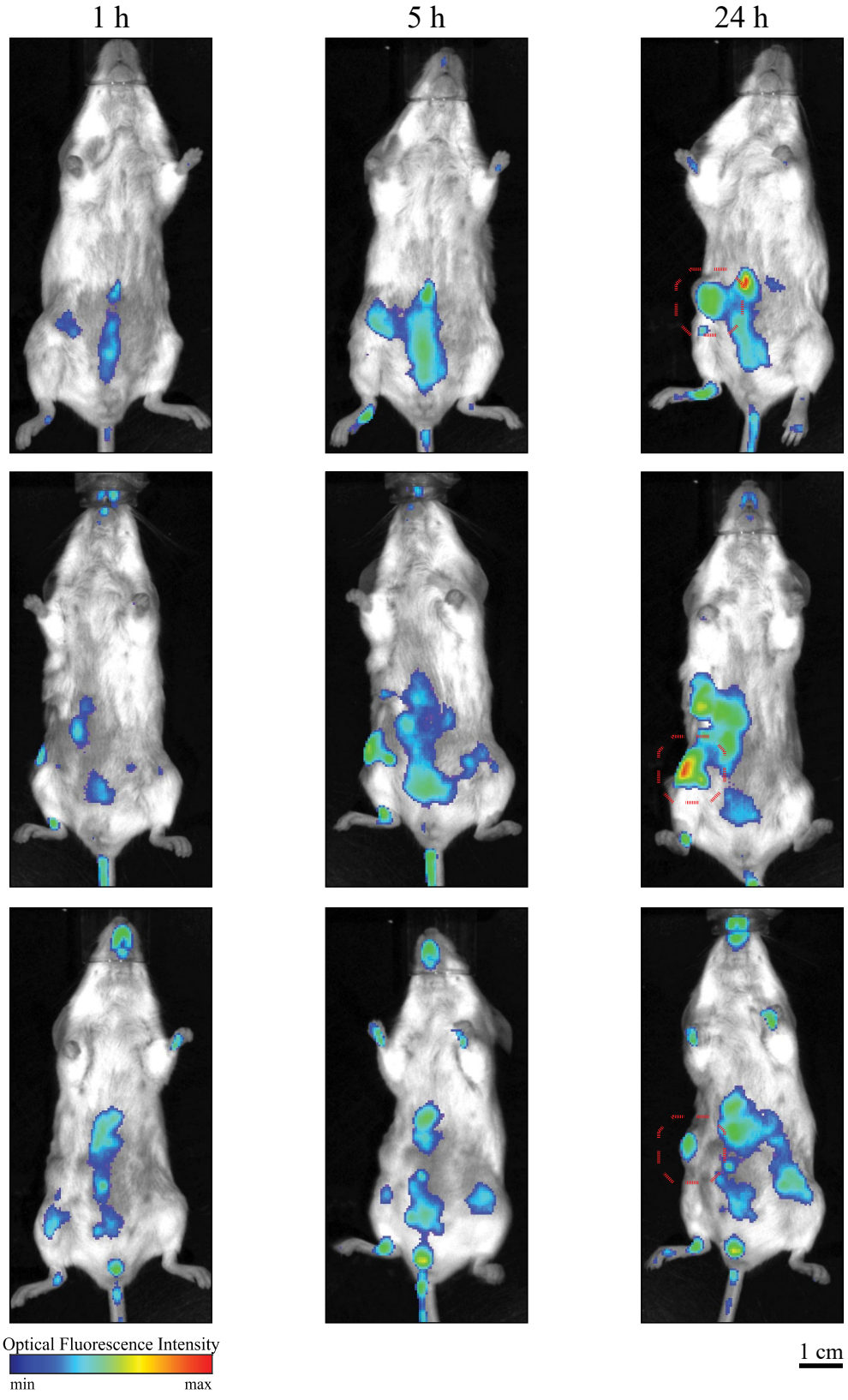

Fig. S9.

In Vivo Optical Fluorescence Imaging. Mice injected with Ru-Lipo were imaged after 1 h, 5 h, and 24 h. Optical fluorescence signal overlaid on photograph. Tumor area is indicated with a dashed red circle. Scale bar, 1 cm.