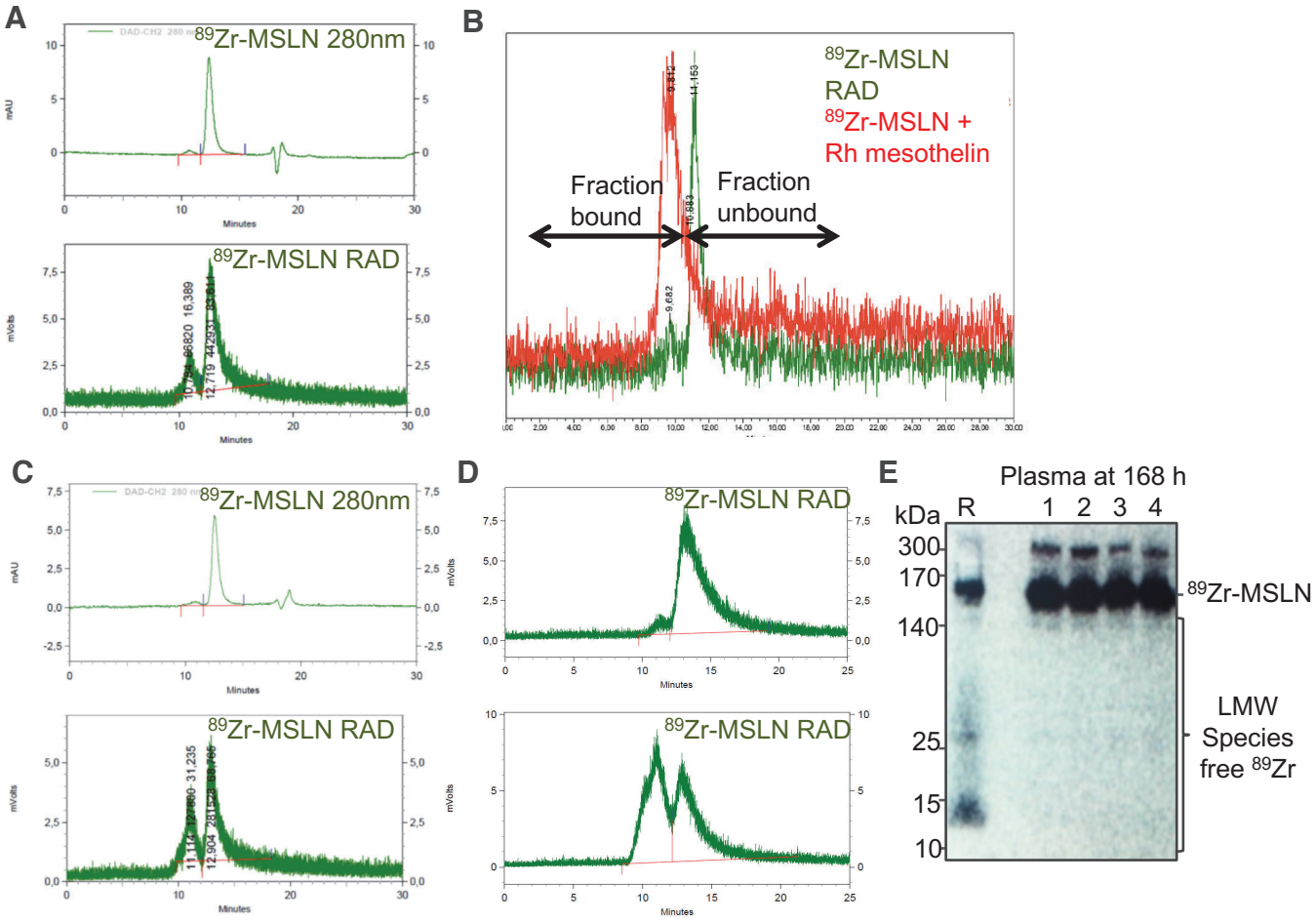
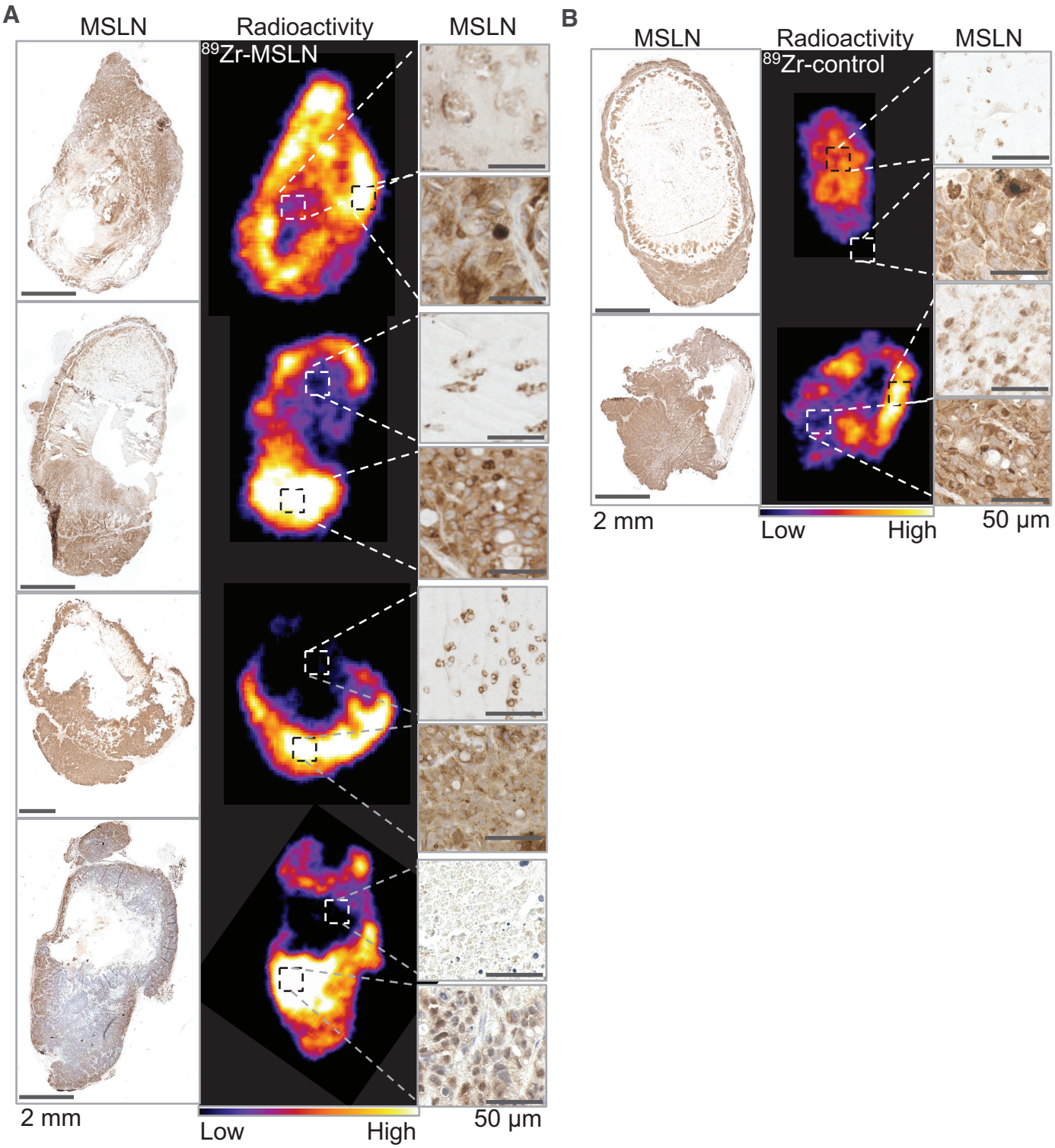


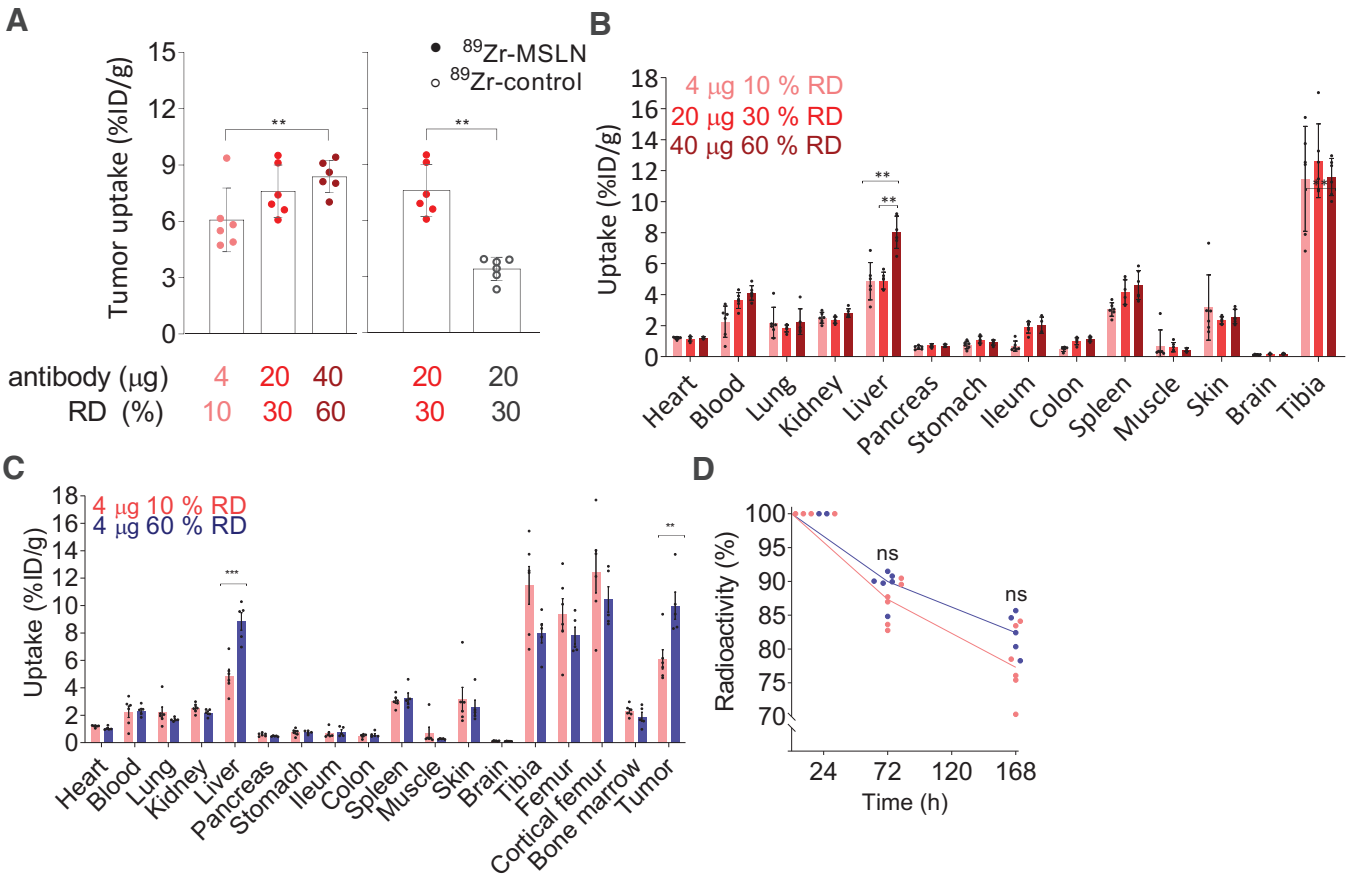
Supplemental Figure 1. ^{89}Zr-MSLN quality control

Ultra-performance liquid chromatography data of **A** optimized ^{89}Zr-MSLN, 4% ± 1% antibody dimers and 15% ± 2% radiolabeled dimers (n = 6) **B** immunoreactive fraction: 0.8 **C** 20 μg ^{89}Zr-MSLN preparation with 30% radioactive dimers **D** ^{89}Zr-MSLN with 10% vs 60% radioactive dimers (at 280 nm ≤5 % data not shown). On y-axis arbitrary units at 280 nm and millivolts at radioactivity detection. **E** *Ex vivo* tracer integrity of ^{89}Zr-MSLN in plasma 168 h pi (n = 4), determined by SDS PAGE, detected by autoradiography, R is ^{89}Zr-MSLN 7 days at RT, including free ^{89}Zr. LMW: low molecular weight. R: reference RAD: radioactivity detection Rh: recombinant human.



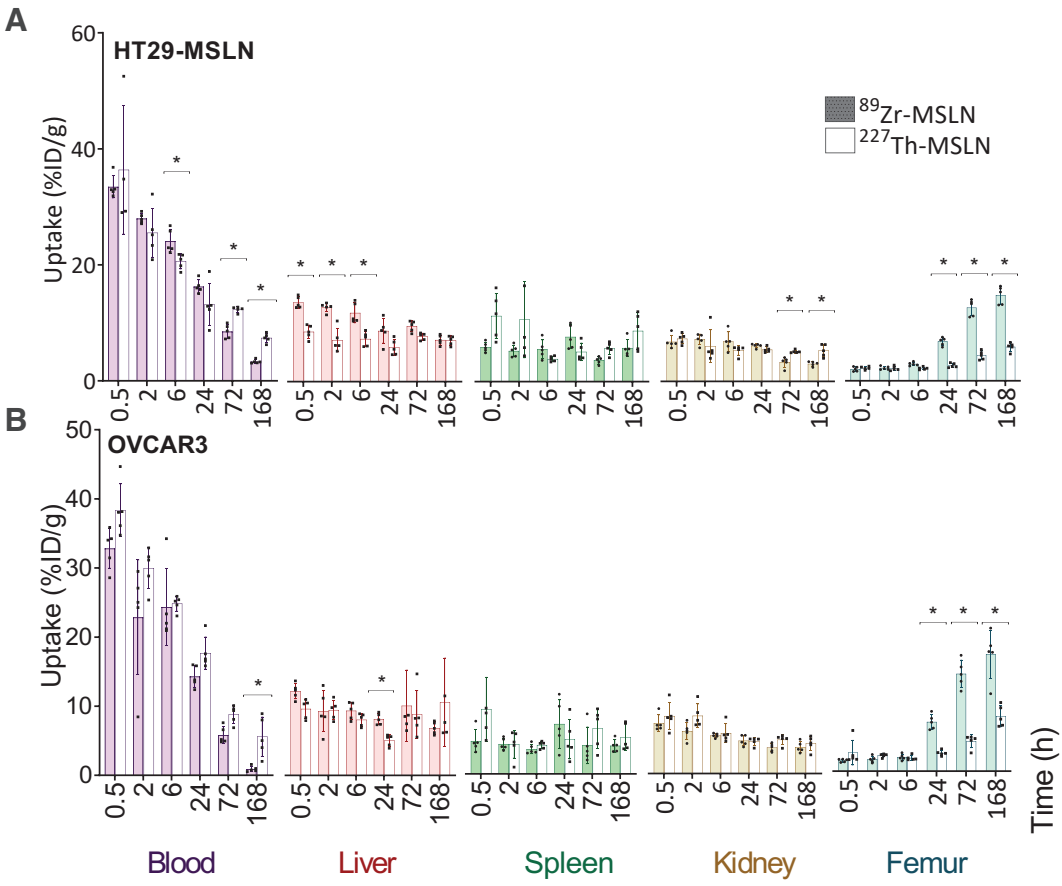
Supplemental Figure 2. Intratumoral ^{89}Zr-MSLN distribution

Mesothelin immunohistochemistry and autoradiography of HT29-MSLN formalin-fixed, paraffin-embedded tumor sections, that received **A** ^{89}Zr-MSLN or **B** ^{89}Zr-control. Mesothelin immunohistochemistry and autoradiography are performed on the same tumor section. Radioactivity is simultaneously scaled in A and B from high to low ^{89}Zr signal intensity. MSLN, Mesothelin.



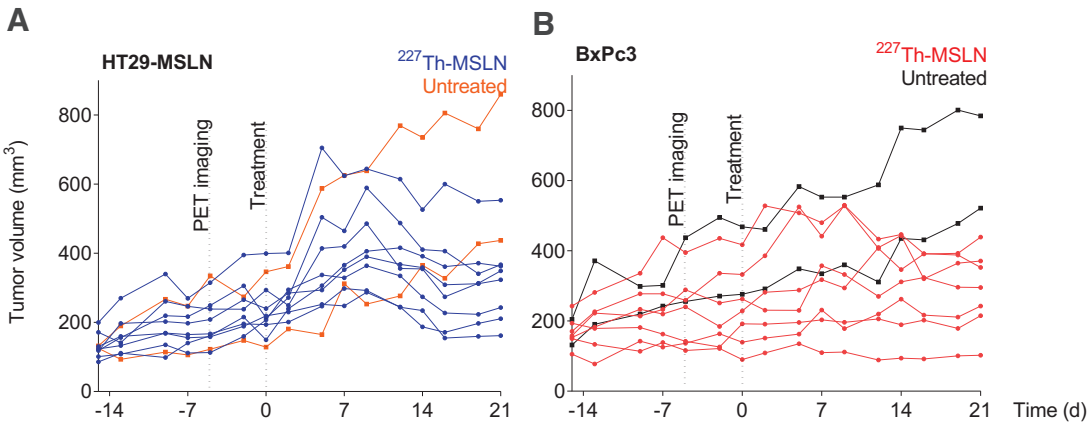
Supplemental Figure 3. Dose- and dimer-effect of ^{89}Zr-MSLN on tumor and healthy tissue uptake

In BxPc3 tumor-bearing mice at 168 h pi **A** *Ex vivo* tumor uptake of 4 µg 10% RD, 20 µg 30% RD, 40 µg 60% RD ^{89}Zr-MSLN and 20 µg 30% ^{89}Zr-control. **B** *Ex vivo* biodistribution of 4 µg 10% RD, 20 µg 30% RD and 40 µg 60% RD ^{89}Zr-MSLN. **C** *Ex vivo* biodistribution of 4 µg ^{89}Zr-MSLN and **D** *in vivo* tracer kinetics, expressed as radioactivity, corrected for decay, with 10% and 60% radioactive dimers. Uptake in tumor and healthy tissues is presented as percentage of injected dose per gram tissue (%ID/g), shown as mean ±SD, including single data points. ***: P <0.001 **: P < 0.01, with Bonferroni conrrection when comparing doses (A). RD: radioactive dimers.

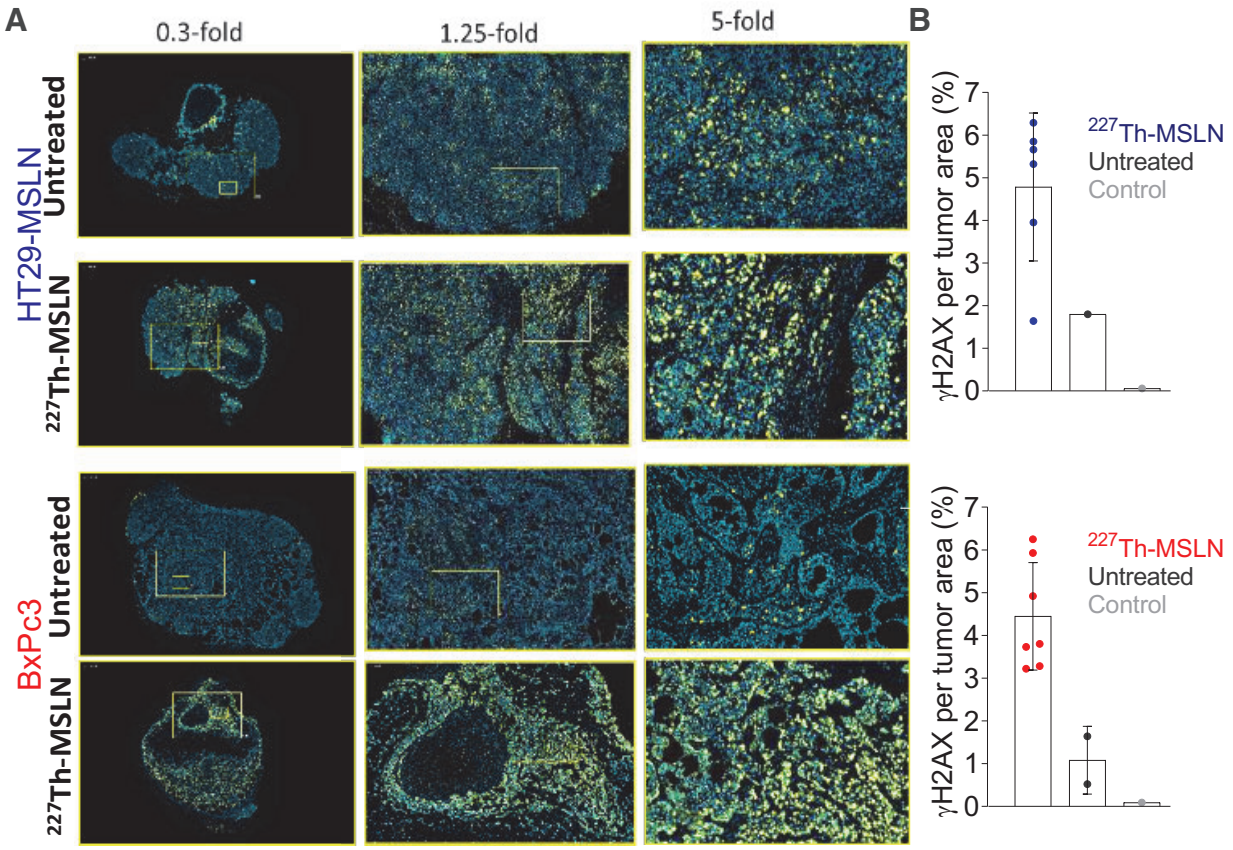


Supplemental Figure 4. ***Ex vivo*** **biodistribution ^{89}Zr-MSLN vs ^{227}Th-MSLN**

in **A** HT29-MSLN tumor-bearing mice and **B** OVCAR3 tumor-bearing mice. Blood levels and uptake in kidney, liver, spleen and intact femur of 20 µg ^{89}Zr-MSLN (0.20 MBq) vs 20 µg ^{227}Th-MSLN (0.015 MBq) at 0.5, 2, 6, 24, 72, and 168 h. Data is presented as mean percentage injected dose per gram tissue (%ID/g) ±SD, including single data points. *: $P < 0.05$ with Bonferroni correction.



Supplemental Figure 5. Absolute tumor growth after ^{227}Th-MSLN treatment of **A** HT29-MSLN tumor-bearing mice (n = 8) and **B** BxPc3 tumor-bearing mice (n = 7) treated with 0.75 mg/kg 500 kBq/kg ^{227}Th-MSLN and n = 2 untreated mice per model. Tumor volumes expressed as mm^3.



Supplemental Figure 6. γH2AX expression in tumors of mice treated with ^{227}Th-MSLN

A γ H2AX immunofluorescence, marking double-strand DNA breaks, in HT29-MSLN and BxPc3 tumors of 0.75 mg/kg 500 kBq/kg ^{227}Th-MSLN treated (n = 6-7 per group) and untreated mice (n = 1-2 per group) harvested 21 days after injection **B** and quantification expressed as % γH2AX per tumor area. Data are mean ±SD, including single data points. Control: monoclonal mouse IgG2a antibody staining control. γH2AX: gamma H2A histone family member X.

γH2AX immunofluorescence

DNA double-strand breaks were detected with immunofluorescence using a human-specific gamma H2A histone family member X (γH2AX) antibody (Cell Signaling, Clone JBW301, mouse), and a monoclonal mouse IgG2a antibody, clone DAK-GO5 (DAKO) was used as control (dilution 1:2000). Sections were exposed to a Cy3-labeled anti-murine-reactive antibody (Perkin Elmer; Opal™ 4-Color Fluorescent IHC Kit). Tumor sections were counter-stained using DAPI. γH2AX foci were quantified using the HS Analysis Webkit tool (HS Analysis; Karlsruhe Institute of Technology, Germany).

Supplemental Table 1: ^{89}Zr-MSLN development for *in vivo* studies: critical conditions

Optimal conditions ^{89}Zr-MSLN production:	
Buffer exchange method	PD gravity filtration with HEPES 0.5 M pH 6.7
^{89}Zr labeling conditions	0.1 mg/mL MSLN-3,2-HOPO concentration 250-500 MBq/mg specific activity In HEPES buffer
Purification method	PD10 gravity filtration purification, elution with: 10 mM histidine 130 mM glycine buffer pH 7.4
<u>Avoid</u> the following conditions to limit radioactive dimer formation:	
Buffer exchange method	Ultracentrifugation (Vivaspin) or NaCl 0.9%
^{89}Zr labeling condition	> 0.1 mg/mL MSLN-HOPO concentration < 200 MBq/mg specific activity Buffers: • Ammonium acetate pH 7 • Ammonium acetate pH 5.5 • Citrate buffer 30 mM • Addition of cold ^{89}Zr
Purification method	Purification by ultracentrifugation (Vivaspin) PD purification with: • NaCl 0.9% • HEPES 0.5 M pH 7 • EDTA addition before purification • Gentisic acid • Tris buffer pH 8.5 • Sodium phosphate buffer pH 7 • Sodium phosphate buffer pH 8.5 • Histidine glycine 10/130 mM pH 8.5 • Glucose 5% • Arginine glycine 50/50 mM pH 8.5

PD: protein desalting, EDTA: ethylenediamine tetraacetic acid.

Supplemental Table 2: ^{89}Zr-MSLN and ^{227}Th-MSLN batches for *in vivo* studies

	^{89}Zr-MSLN				^{89}Zr-control	^{227}Th-MSLN
Antibody dose (µg)	4	4	20	40	20	20
Radioactive dose (MBq)	1	1	3	3	4	0.015
Radiolabeling efficiency (%)	60	60	64	92	76	99
Antibody concentration (mg/ml)	0.1	0.1	0.2	0.4	0.2	0.2
Specific activity (MBq/mg)	400	400	250	125	250	0.7
Radiochemical purity (%)	99	99	97	99	98	99
Radioactive dimers (%)	10	60	30	60	30	<10
Total antibody dimers (%)	<5	5	5	5	5	<5

Batches of 4 μg and 40 μg ^{89}Zr-MSLN were injected in the BxPc3 tumor-bearing mice (Suppl. Fig. 3), 20 μg ^{89}Zr-MSLN and ^{89}Zr-control were injected in HT29-MSLN tumor-bearing mice (Fig. 1). The 20 μg ^{227}Th-MSLN was injected in HT29-MSLN tumor-bearing mice (Fig. 5).