Supplemental Data

SI. METHODS

Sample preparation for gelatin zymography

Cell assays.

Confluent HT-1080 cells were washed with Dulbecco's PBS and incubated in serum-free MEM containing 20 nM phorbol 12-myristate 13-acetate (PMA) for 48h. The medium was centrifuged (1,000 rpm, 5 min) and supernatant was stored at -80 °C until zymography analysis. Typically, 20 µL supernatant was used per analysis.

Tissue homogenates.

After γ-counting of the various organs and tissues of tumor-bearing mice (n=3), the tissue samples were transferred to a 2 mL eppendorf tube and weighed. A tungsten bead (5 mm) and 10 volumes of 50 mM Tris, 200 mM NaCl, 10 mM $CaCl_2$, and 10 µM $ZnCl_2$ at pH 7.5 were added. The tissues were homogenized at 30 Hz for 5 min using a tissue lyser (Qiagen), mixed for 4h at 4 °C and then centrifuged at 10,000 rpm for 5 min. The supernatants were aliquoted and stored at -80 °C until zymography analysis. Typically, 0.9 µL tissue supernatant (from 0.08 µg tissue) was used per analysis.

Blood samples.

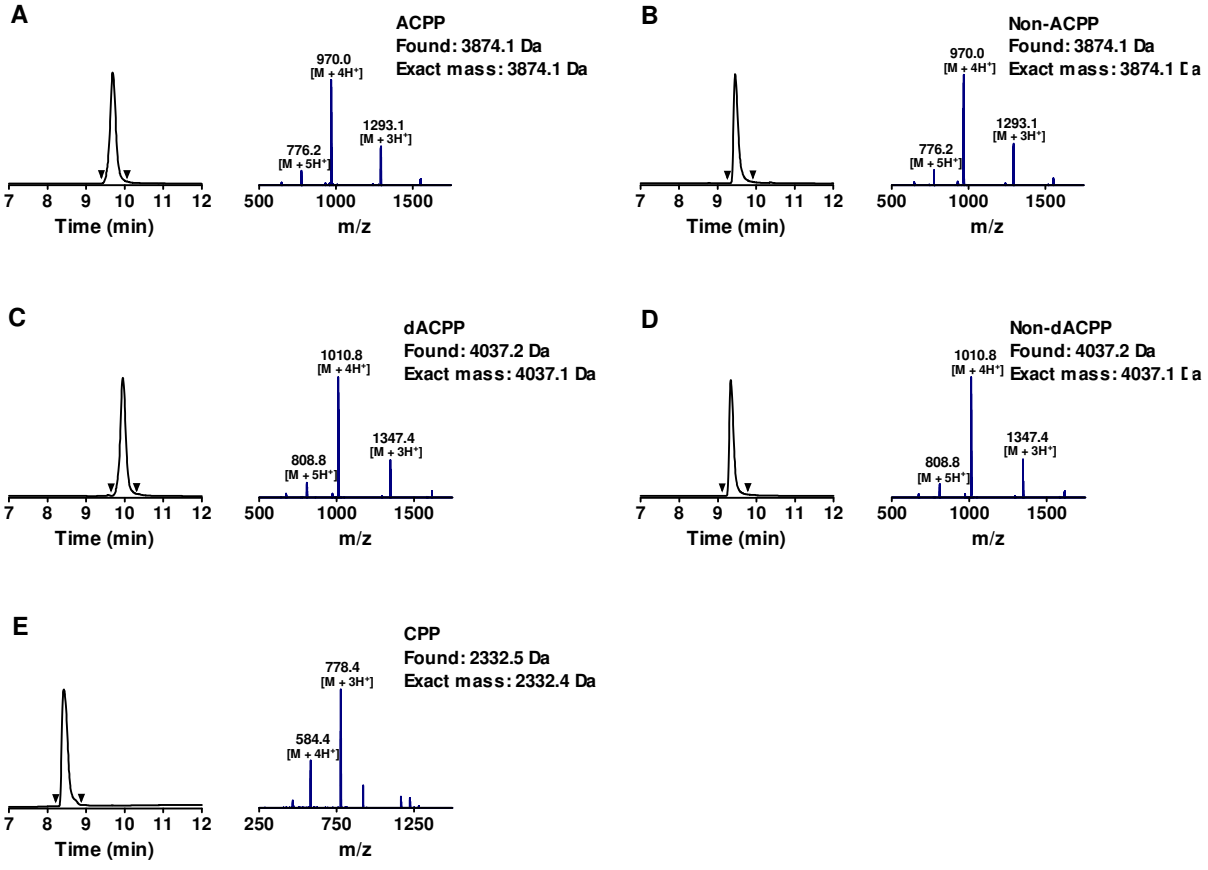
Fresh mouse blood (50 µL from vena saphena) was centrifuged at 5000 rpm for 5 min. Serum was pipetted off and stored at -80 °C until zymography analysis. Typically, 2.0 µL serum was used per analysis.

Gelatin zymography

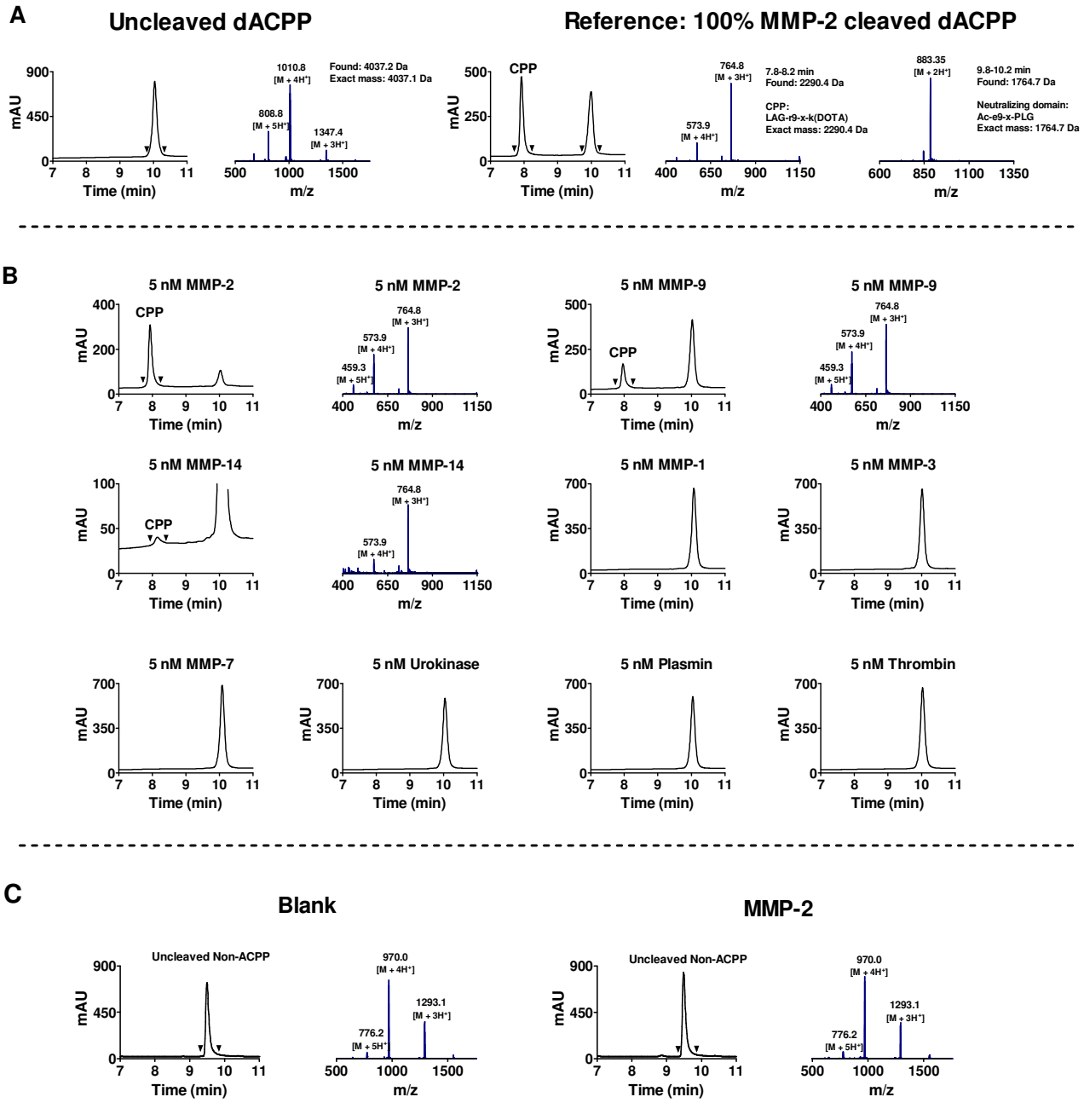
Samples were analyzed on 10% SDS-PAGE gel containing 0.1% w/v gelatin A (TetraCell, Biorad). After running the gel at 150V, it was washed with MilliQ water, incubated for 30 min in 2.5% Triton-X (60 rpm), washed with MilliQ water, and incubated in 50 mM Tris, 200 mM NaCl, 5 mM $CaCl_2$, 0.1% w/v NaN_3, and 0.02% w/v Brij-35 at pH 7.6 and 37 ^{o}C for 68h. The gel was stained for 2h with 0.25% Coomassie Blue in 60% v/v MilliQ water, 30% v/v Methanol, 10% v/v Acetic acid, destained for 2h with 67.5% v/v MilliQ water, 25% v/v Methanol, 7.5% v/v Acetic acid and imaged (Epson Perfection V700 Photo scanner). Gelatinatic activity showed up as clear bands against a dark background.

Data analysis

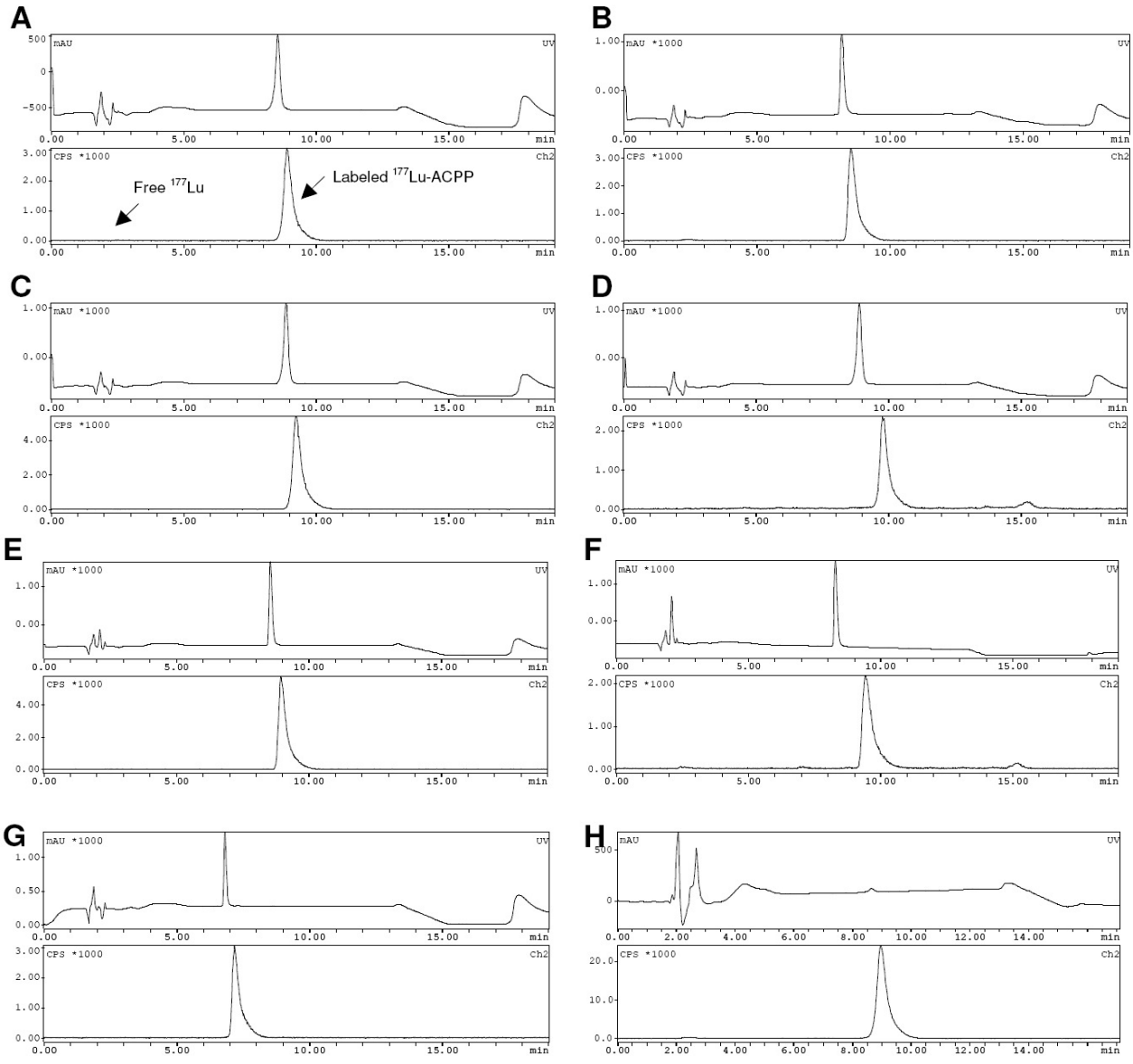
Gelatin zymogram images were analyzed by ImageJ to approximately determine the MMP concentration. The color of each pixel was converted to a pixel value, ranging from 0 (black) to 255 (white). Blue background pixels belonging to stained gelatin displayed pixel values typically in the range of 100-145. Pixels with a pixel value higher than 155 were categorized as MMP. To determine the MMP concentration of a sample, the pixel values of all MMP-categorized pixels were summed up and compared to the reference sample (0.13 ng MMP-2).



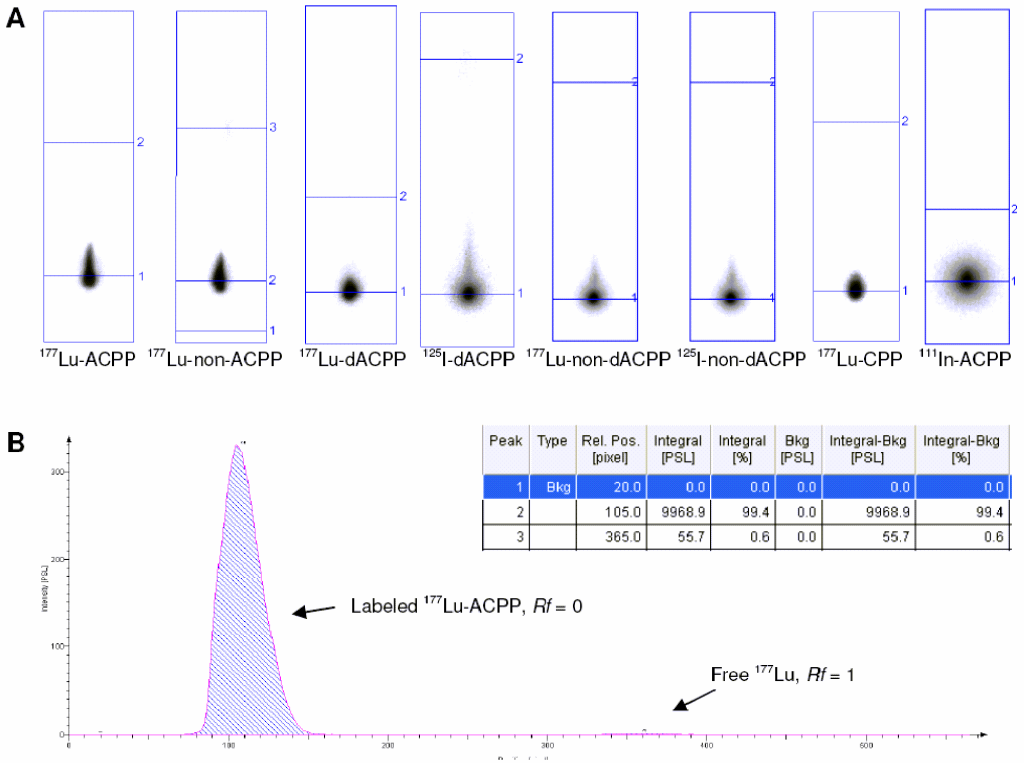
Supplemental Fig. 1. LC-MS spectra of (A) Ac-e_9-*x*-PLGLAG-r_9-*x*-k(DOTA)-NH_2 (ACPP), (B) Ac-e_9-*x*-LALGPG-r_9-*x*-k(DOTA)-NH_2 (non-ACPP), (C) Ac-y-e_9-x-PLGLAG-r_9-x-k(DOTA)-NH2 (dACPP), (D) Ac-y-e_9-x-LALGPG-r_9-x-k(DOTA)-NH_2 (non-dACPP), and (E) Ac-LAG-r_9-*x*-k(DOTA)-NH_2 (CPP). The left and right graphs show the UV absorbance chromatogram at 210 nm and the mass spectrum of the UV-peak bracketed by the arrowheads, respectively. Buffer A: 0.1% trifluoroacetic acid (TFA) in MilliQ water. Buffer B: 0.1% TFA in acetonitril.



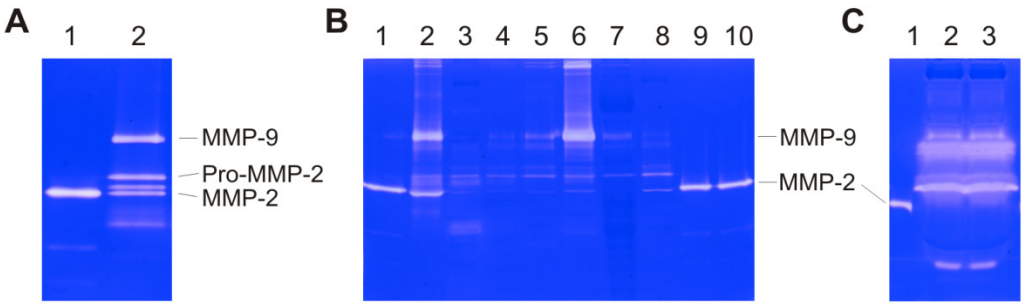
Supplemental Fig. 2. (A) LC-MS spectra of dACPP (0.1 mM) before and 1h after incubation with MMP-2 (30 nM) to yield 100% cleaved dACPP. (B) Enzyme assay: LC-MS spectra of dACPP (0.1 mM) 1h after incubation with 5 nM of either MMP-1, MMP-2, MMP-3, MMP-7, MMP-9, MMP-14, Urokinase, Plasmin, or Thrombin (representative of three measurements). (C) Non-ACPP (0.1 mM) before and 1h after incubation with MMP-2 (5 nM). Buffer A: 0.1% TFA in MilliQ water. Buffer B: 0.1% TFA in acetonitril.



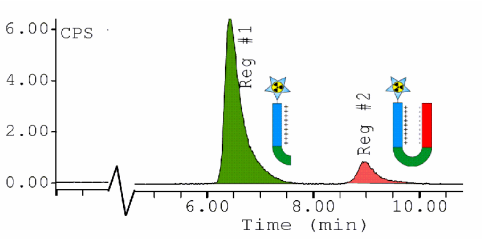
Supplemental Fig. 3. Radio-HPLC spectra of the radio labeled peptides (A) ^{177}Lu-ACPP, (B) ^{177}Lu-non-ACPP, (C) ^{177}Lu-dACPP, (D) ^{125}I-dACPP, (E) ^{177}Lu-non-dACPP, (F) ^{125}I-non-dACPP, (G) ^{177}Lu-CPP, and (H) ^{111}In-ACPP. The upper panel shows the UV absorbance chromatogram at 212 nm. γ-radiation was monitored by a γ-detector and is shown in the lower panel. Used gradient: 3.6 %B/min, starting with 5 %B at t=0 min. Buffer A: 0.1% TFA in MilliQ water. Buffer B: 0.1% TFA in acetonitrile. ^{111}In-ACPP was labeled at SPECT imaging dose (1 MBq ^{111}In/nmol probe).



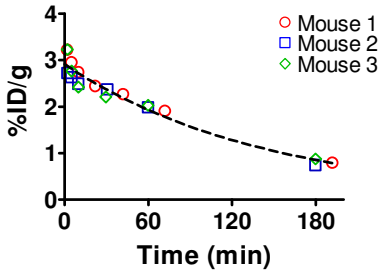
Supplemental Fig. 4. Instant silica thin layer chromatography of radio labeled peptides. (A) From left to right: ^{177}Lu-ACPP, ^{177}Lu-non-ACPP, ^{177}Lu-dACPP, ^{125}I-dACPP, ^{177}Lu-non-dACPP, ^{125}I-non-dACPP, ^{177}Lu-CPP, and ^{111}In-ACPP. Free ^{177}Lu/^{125}I runs with mobile phase (R_f = 1.0), while ^{177}Lu/^{125}I labeled peptides do not run (R_f = 0). (B) Integration of signal for ^{177}Lu-ACPP revealed 99.4% radiochemical purity (typically >99.0%). Radiochemical purities found for other probes: 99.5% for ^{177}Lu-non-ACPP (typically >99.0%), 100% for ^{177}Lu-dACPP (typically >99%), 96.5% for ^{125}I-dACPP (typically >96%), 100% for ^{177}Lu-non-dACPP (typically >99%), 98.9% for ^{125}I-non-dACPP (typically >96%), 100% for ^{177}Lu-CPP (typically >99%), and 100% for ^{111}In-ACPP (typically >97%).



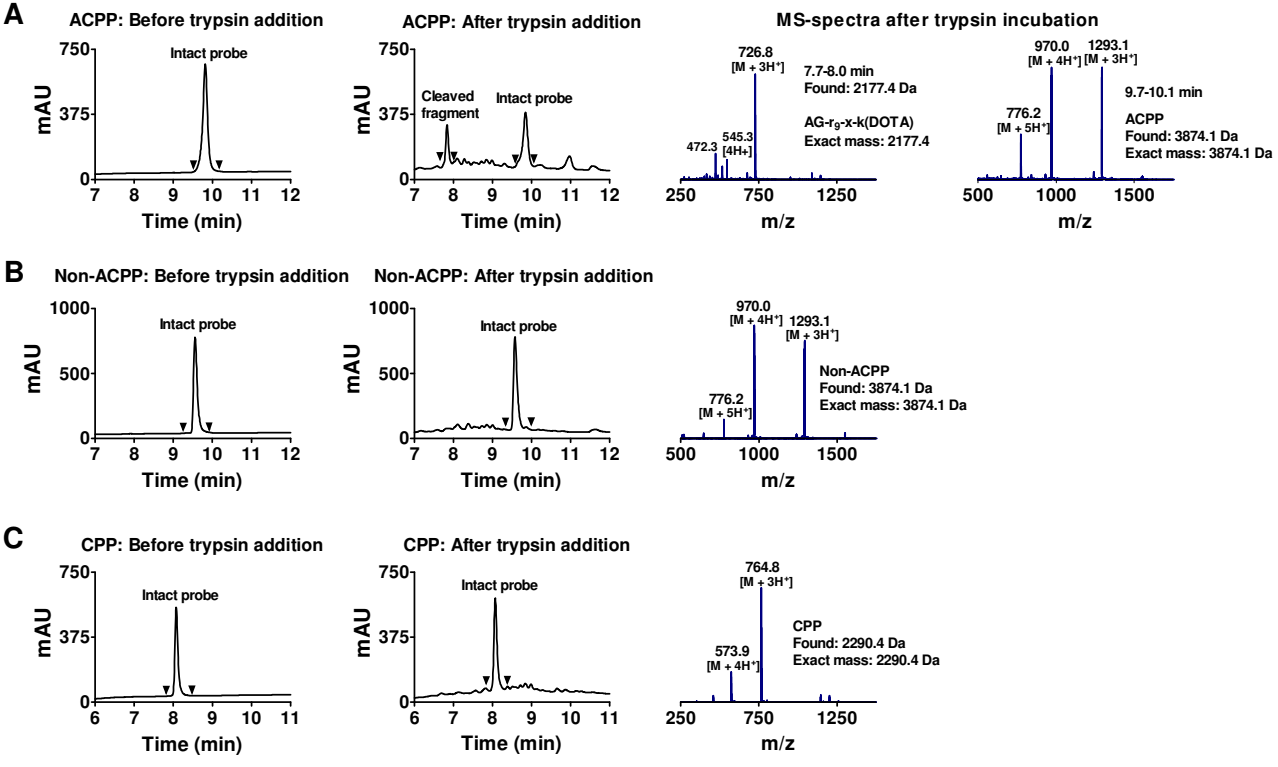
Supplemental Fig. 5. Gelatin zymography on 10% SDS-PAGE gel containing 0.1% w/v gelatin A. (A) Analysis of 20 µL of serum-free medium of PMA stimulated HT-1080 cells (48h incubation) (lane 2). Active MMP-2 (0.13 ng) was loaded as reference (lane 1). (B) Analysis of tissue homogenates (0.08 µg, representative of three mice). Lane 1 = active MMP-2 (0.13 ng), lane 2 = tumor, lane 3 = muscle, lane 4 = heart, lane 5 = lung, lane 6 = spleen, lane 7 = liver, lane 8 = bladder, lanes 9 and 10 = active MMP-2 (0.13 ng). (C) Analysis of mouse serum. Lane 1 = active MMP-2 (0.13 ng), lane 2 = 2.0 µL serum from tumor-free mice, lane 3 = 2.0 µL serum from tumor-bearing mice.



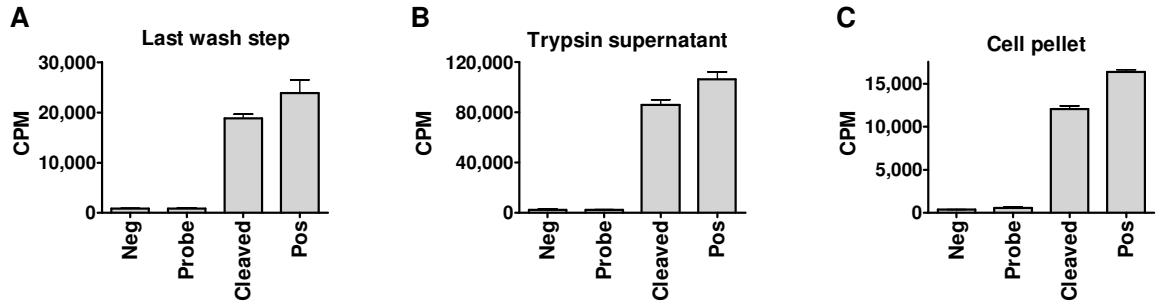
Supplemental Fig. 6. Activation of ^{177}Lu-ACPP by active MMP-2 analyzed by radio-HPLC. After 3h of incubation 85% of ^{177}Lu-ACPP was activated as was calculated from AUC values.



Supplemental Fig. 7. Blood kinetic measurements of 60 nmol ^{177}Lu-CPP (n=3). The data are presented as mean %ID/g ± SD.



Supplemental Fig. 8. LC-MS spectra of (A) Ac-e$_9$-*x*-PLGLAG-r$_9$-*x*-k(DOTA)-NH$_2$ (ACPP), (B) Ac-e$_9$-*x*-LALGPG-r$_9$-*x*-k(DOTA)-NH$_2$ (non-ACPP), and (C) LAG-r$_9$-*x*-k(DOTA)-NH$_2$ (CPP) before and 5 min after incubation with trypsin (0.125 w/v %). The left and right graphs show the UV absorbance chromatogram at 210 nm and the mass spectrum of the UV-peak bracketed by the arrowheads, respectively. Buffer A: 0.1% TFA in MilliQ water. Buffer B: 0.1% TFA in acetonitril.



Supplemental Fig. 9. HT-1080 fibrosarcoma cell assay showing γ-counting results for (A) last Dulbecco's PBS wash fraction, (B) trypsin supernatant, and (C) cell pellet for ^{177}Lu-non-ACPP, uncleaved ^{177}Lu-ACPP, pre-activated (for 85%) ^{177}Lu-ACPP, and ^{177}Lu-CPP after 3h incubation. The experiments were performed in triplicate and the data are presented as mean ± SD. For pre-activated ACPP and CPP a substantial amount of radioactivity was detected in the last Dulbecco's PBS wash step. Most likely, this observation can be explained by detachment of CPP which was bound to the ECM of the cells and not yet internalized.

Probe sensitivity towards trypsin, consequences for *in vitro* cell assay

In *in vitro* cell assays, trypsin digestion is a commonly used method to harvest cells. To test whether trypsin is able to cleave our probes, we incubated ACPP, non-ACPP, and CPP with trypsin (0.1 mM peptide in 50% v/v Dulbecco's PBS and 50% v/v trypsin-EDTA (0.25% w/v)) at 37 °C. After 5 min, TFA (10% v/v) was added to quench tryptic activity and samples were analyzed by LC-MS. A substantial amount of ACPP (~30%) was cleaved by trypsin, while under similar conditions no cleavage/degradation occurred for non-ACPP and CPP (Supplemental Fig. 8). ACPP was cleaved between the amino acids leucine and alanine present in the MMP-2 cleavable linker PLGLAG. To exclude trypsin-mediated ACPP activation in our *in vitro* cell assay, the cells incubated with the probes were carefully washed 5-times with Dulbecco's PBS before we harvested the cells by trypsin. These wash steps ensured that the soluble probes were removed before trypsin digestion (last wash fraction contained <0.05% radioactivity compared to incubation medium for non-ACPP and intact ACPP). Our data showed that the cellular uptake of the negative control and trypsin sensitive intact probe was similar, indicating that there is no significant role of trypsin on ACPP activation in this experiment (Supplemental Fig. 9).

MMP concentration in serum and cell culture supernatant

Gelatin zymograms (Supplemental Fig. 5) were analyzed to approximately determine the MMP concentration. We found an MMP concentration of 13 ng/mL MMP for cell culture supernatant, 1.04 ± 0.05 µg/mL for serum from tumor-free mice, and 1.08 ± 0.04 µg/mL for serum from the same mice bearing an HT-1080 xenograft (n=3). The latter results indicate that the presence of an MMP-2 expressing tumor did not result in increased MMP concentration in serum. Our analysis further revealed an ~80-fold higher MMP concentration in serum compared to cell culture supernatant (in which MMPs were too diluted to observe ACPP activation) and may suggest that MMPs present in blood are the presumable source of ACPP activation *in vivo*. We should remark, however, that MMPs can be inhibited by tissue inhibitors of metalloproteinase, and, therefore, the difference in MMP concentration might not relate in a 1:1-fashion to the difference in MMP activity between serum and cell culture supernatant.

Supplemental Table 1: Biodistribution results of 60 nmol ^{177}Lu-ACPP and ^{177}Lu-non-ACPP (6h and 24h), and ^{177}Lu-CPP in tumor-bearing mice 24h post injection. The data are presented as mean %ID/g ± SD.

	^{177}Lu-ACPP, 6h, n=3	^{177}Lu-non-ACPP, 6h, n=3	^{177}Lu-ACPP, 24h, n=6	^{177}Lu-non-ACPP, 24h, n=6	^{177}Lu-CPP, 24h, n=6	Ratio: ACPP/non-ACPP, 6h	Ratio: ACPP/non-ACPP, 24h	Ratio: ACPP/CPP, 24h
Blood	0.04 ± 0.02	0.00 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.03 ± 0.01	8.1	1.9	0.2
Tumor	1.90 ± 0.71	0.31 ± 0.14	2.23 ± 1.09	0.37 ± 0.27	3.37 ± 1.26	6.1	6.0	0.7
Muscle	0.48 ± 0.18	0.03 ± 0.01	0.46 ± 0.20	0.04 ± 0.01	0.53 ± 0.10	14.0	12.0	0.9
Heart	0.33 ± 0.03	0.04 ± 0.01	0.25 ± 0.08	0.03 ± 0.01	0.74 ± 0.18	9.1	7.6	0.3
Lung	0.82 ± 0.13	0.15 ± 0.05	0.70 ± 0.20	0.15 ± 0.03	1.63 ± 0.08	5.5	4.6	0.4
Spleen	2.79 ± 0.39	0.36 ± 0.06	4.00 ± 1.62	0.43 ± 0.06	8.07 ± 1.03	7.8	9.3	0.5
Liver	17.0 ± 2.62	2.16 ± 0.36	20.3 ± 4.65	2.89 ± 0.33	41.6 ± 4.21	7.9	7.0	0.5
Kidney (left)	52.2 ± 1.23	84.7 ± 17.9	52.4 ± 10.9	74.4 ± 11.5	39.0 ± 6.39	0.6	0.7	1.3
Fat	0.27± 0.15	0.03 ± 0.03	0.23 ± 0.02	0.04 ± 0.02	0.51 ± 0.20	9.8	6.1	0.5
Bladder	0.55 ± 0.08	0.13 ± 0.06	0.45 ± 0.09	0.18 ± 0.09	1.00 ± 0.13	4.3	2.6	0.5
Colon (empty)	0.64 ± 0.18	0.13 ± 0.10	0.58 ± 0.20	0.08 ± 0.01	1.52 ± 0.20	5.0	7.4	0.4
Thigh bone	2.66 ± 0.20	0.50 ± 0.07	2.66 ± 0.68	0.67 ± 0.21	3.81 ± 0.33	5.3	12.0	0.7
Brain	0.01 ± 0.00	0.00 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.03 ± 0.00	5.1	7.2	0.5

Supplemental Table 2: Tumor-to-tissue ratios for ^{177}Lu-ACPP and ^{177}Lu-non-ACPP (6h and 24h), and ^{177}Lu-CPP in tumor-bearing mice. The data are presented as mean ± SD.

Ratio	^{177}Lu-ACPP, 6h, n=3	^{177}Lu-non-ACPP, 6h, n=3	^{177}Lu-ACPP, 24h, n=6	^{177}Lu-non-ACPP, 24h, n=6	^{177}Lu-CPP, 24h, n=6
Tumor/muscle	4.8 ± 3.3	9.7 ± 5.8	5.2 ± 2.5	9.5 ± 4.5	6.4 ± 2.1
Tumor/heart	5.9 ± 2.3	9.6 ± 4.5	10.2 ± 5.4	11.3 ± 7.1	5.1 ± 3.1
Tumor/lung	2.2 ± 0.6	2.2 ± 1.0	3.5 ± 1.8	2.4 ± 1.6	2.1 ± 0.8
Tumor/spleen	0.7 ± 0.3	0.9 ± 0.5	0.7 ± 0.4	0.9 ± 0.7	0.4 ± 0.2
Tumor/liver	0.11 ± 0.03	0.15 ± 0.06	0.11 ± 0.05	0.13 ± 0.10	0.08 ± 0.04
Tumor/kidney	0.04 ± 0.01	0.004 ± 0.002	0.04 ± 0.02	0.005 ± 0.004	0.09 ± 0.04

Supplemental Table 3: Biodistribution results of 60 nmol ^{177}Lu/^{125}I-dACPP and ^{177}Lu/^{125}I-non-dACPP in tumor-bearing mice 24h post injection (n=6) and tumor-free mice (n=4). The data are presented as mean %ID/g ± SD.

	^{177}Lu/^{125}I-dACPP in tumor-bearing mice, n=6			^{177}Lu/^{125}I-non-dACPP in tumor-bearing mice, n=6			^{177}Lu/^{125}I-dACPP in tumor-free mice, n=4		
	^{177}Lu	^{125}I	Ratio ^{177}Lu/^{125}I	^{177}Lu	^{125}I	Ratio ^{177}Lu/^{125}I	^{177}Lu	^{125}I	Ratio ^{177}Lu/^{125}I
Blood	0.01 ± 0.02	0.01 ± 0.00	ND	0.01 ± 0.00	0.01 ± 0.01	ND	0.02 ± 0.01	0.01 ± 0.00	ND
Tumor	1.65 ± 0.66	0.13 ± 0.03	13.4 ± 6.2	0.52 ± 0.25	0.18 ± 0.06	2.9 ± 1.9	-	-	-
Muscle	0.47 ± 0.18	0.02 ± 0.01	22.1 ± 3.8	0.05 ± 0.02	0.03 ± 0.01	1.4 ± 0.3	0.18 ± 0.06	0.01 ± 0.00	12.9 ± 2.9
Heart	0.23 ± 0.07	0.02 ± 0.00	10.6 ± 4.4	0.04 ± 0.00	0.03 ± 0.00	1.3 ± 0.1	0.32 ± 0.04	0.03 ± 0.00	11.8 ± 1.5
Lung	0.61 ± 0.25	0.05 ± 0.01	11.4 ± 3.3	0.21 ± 0.04	0.06 ± 0.01	3.6 ± 0.1	0.86 ± 0.18	0.05 ± 0.01	16.6 ± 0.8
Spleen	3.23 ± 1.63	0.07 ± 0.02	46.2 ± 18.2	0.54 ± 0.09	0.14 ± 0.01	3.8 ± 0.7	4.81 ± 0.77	0.08 ± 0.01	58.3 ± 12.2
Liver	18.4 ± 3.67	0.25 ± 0.05	77.5 ± 25.8	3.50 ± 0.60	0.68 ± 0.11	5.2 ± 0.8	24.6 ± 1.51	0.19 ± 0.04	130.7 ± 15.2
Kidney (left)	47.5 ± 7.70	17.0 ± 2.97	2.8 ± 0.4	84.0 ± 14.3	50.5 ± 8.84	1.7 ± 0.0	52.1 ± 8.61	18.5 ± 2.46	2.8 ± 0.2
Fat	0.26 ± 0.15	0.02 ± 0.01	ND	0.08 ± 0.09	0.06 ± 0.07	ND	0.31 ± 0.11	0.02 ± 0.01	ND
Bladder	0.46 ± 0.24	0.29 ± 0.25	ND	0.11 ± 0.03	0.16 ± 0.02	ND	0.50 ± 0.07	0.18 ± 0.06	ND
Colon (empty)	0.49 ± 0.20	0.05 ± 0.01	ND	0.08 ± 0.02	0.06 ± 0.01	ND	0.68 ± 0.09	0.05 ± 0.01	ND
Thigh bone	2.57 ± 0.38	0.55 ± 0.11	4.8 ± 1.3	0.87 ± 0.19	0.58 ± 0.09	1.5 ± 0.2	2.75 ± 0.17	0.61 ± 0.13	4.6 ± 0.8
Brain	0.01 ± 0.00	0.00 ± 0.00	ND	0.00 ± 0.00	0.00 ± 0.00	ND	0.01 ± 0.00	0.00 ± 0.00	ND
Stomach (full)	0.22 ± 0.06	0.12 ± 0.06	ND	0.05 ± 0.04	0.08 ± 0.04	ND	0.28 ± 0.12	0.11 ± 0.12	ND
Intestine (full)	0.29 ± 0.09	0.05 ± 0.01	ND	0.06 ± 0.03	0.05 ± 0.02	ND	0.38 ± 0.09	0.10 ± 0.13	ND
Thyroid*	0.12 ± 0.03	1.51 ± 0.45	ND	0.00 ± 0.00	0.78 ± 0.35	ND	0.16 ± 0.07	0.96 ± 0.10	ND

* expressed in %ID