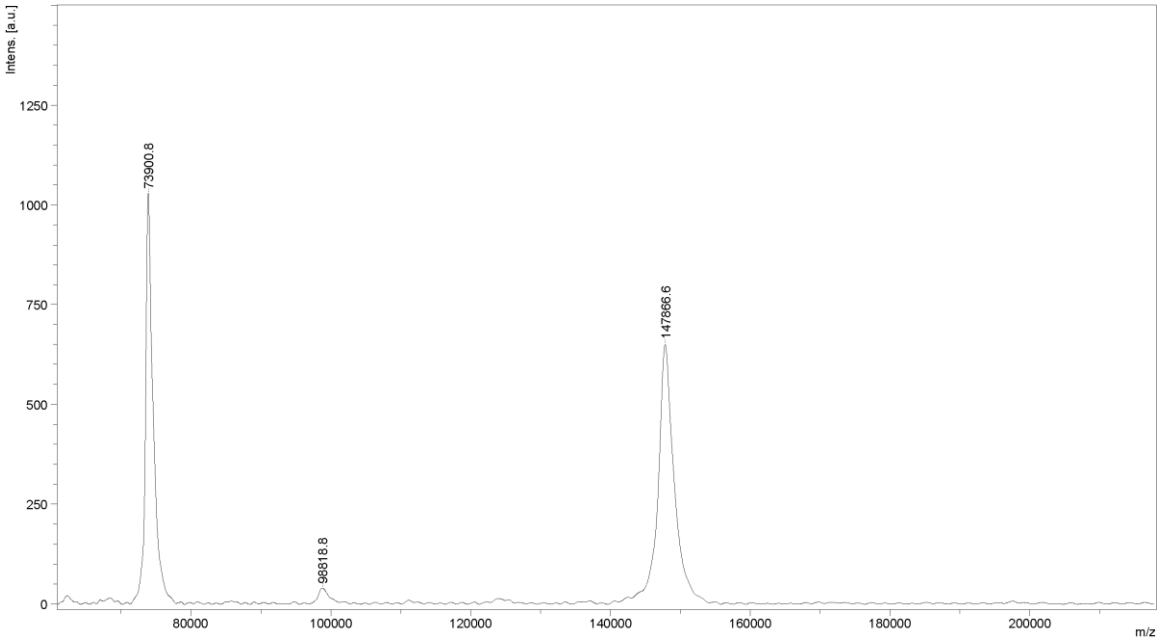
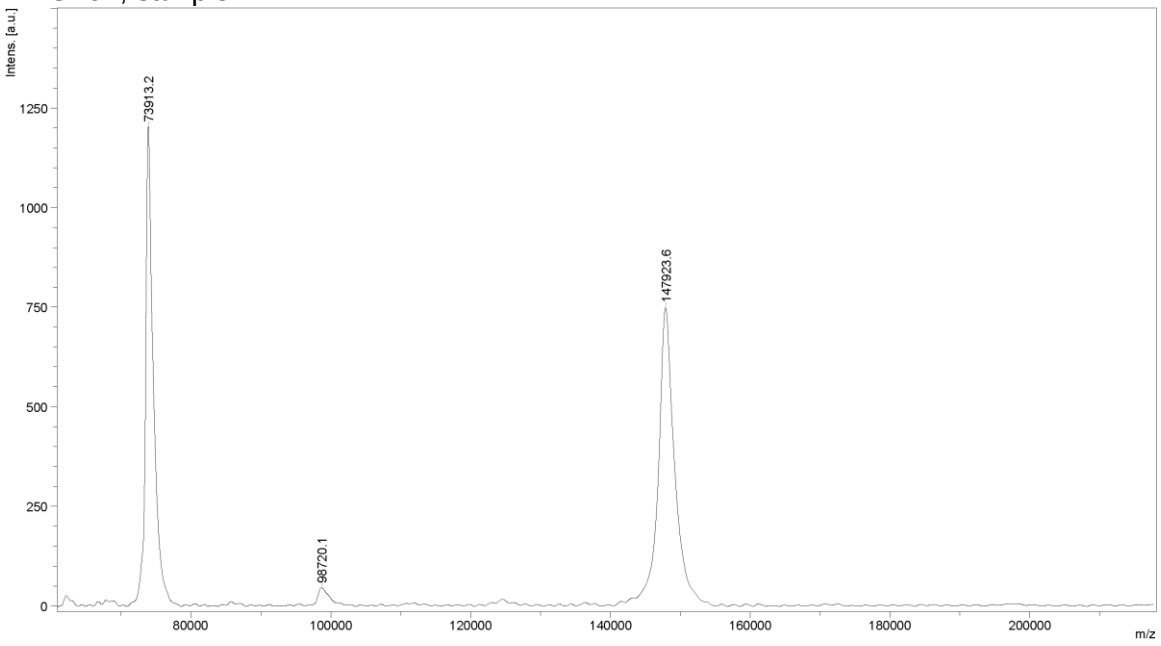
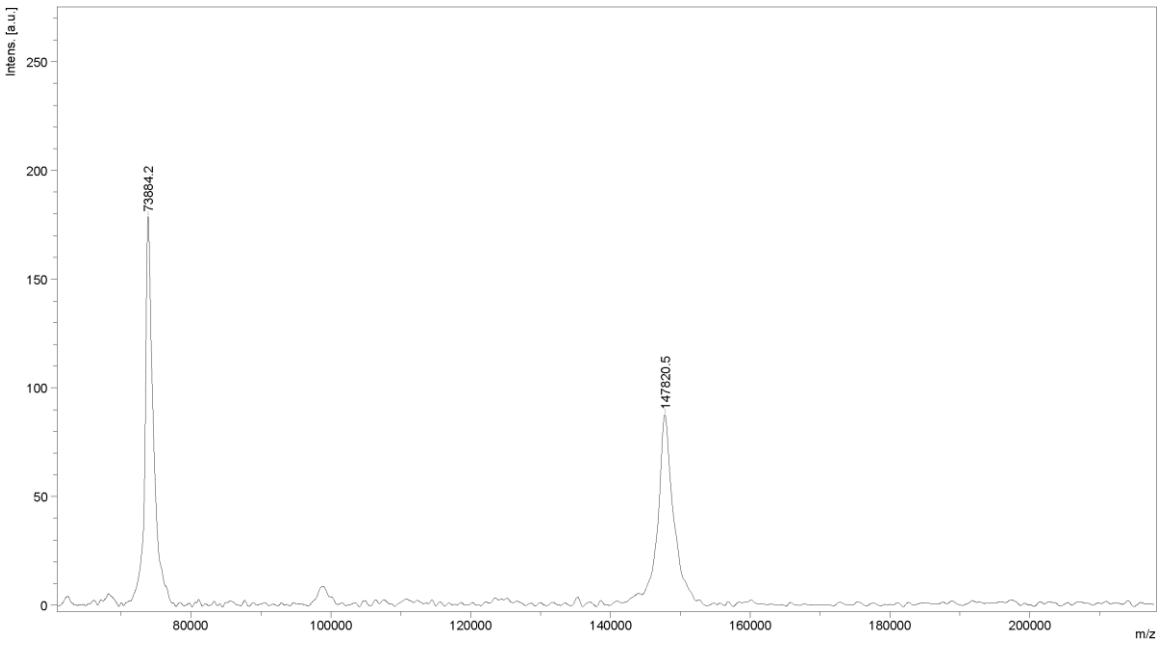


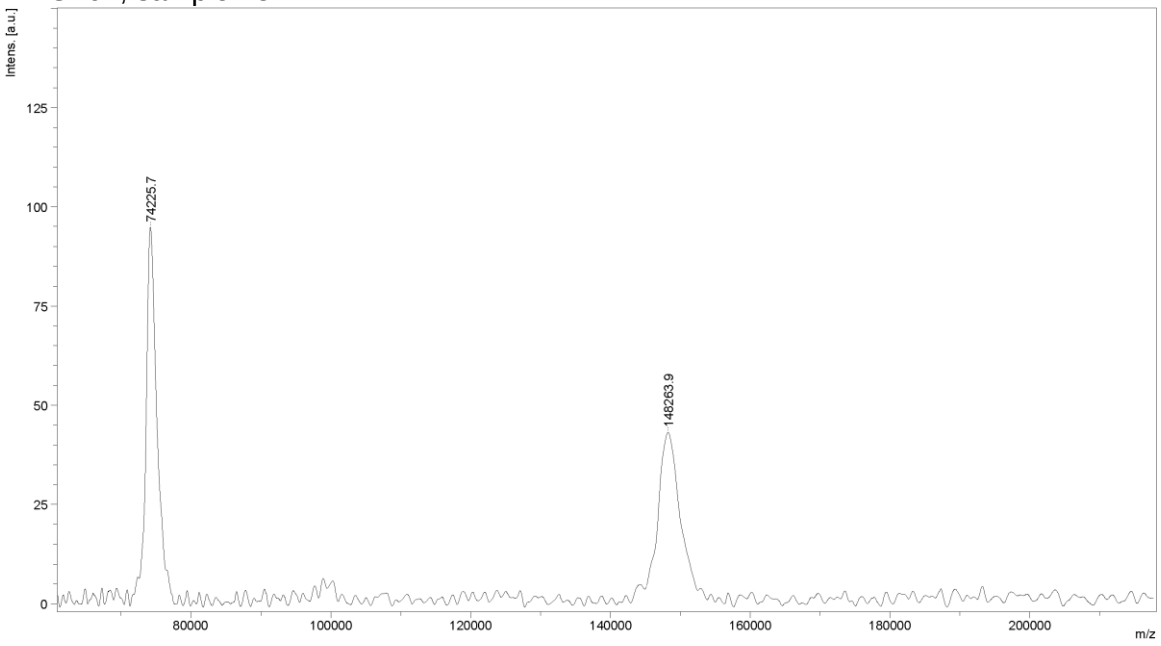

**Supplemental Fig. 1.** MALDI-TOF MS/MS mass spectrometry analysis of unmodified AMG102, sample #1.



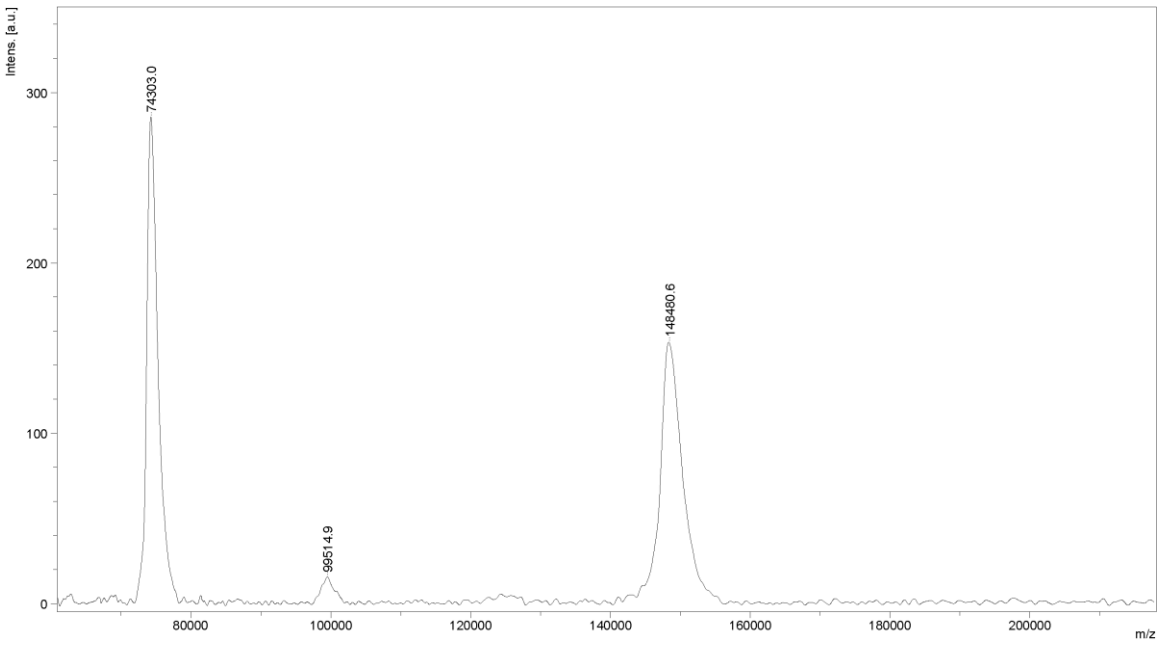

**Supplemental Fig. 2.** MALDI-TOF MS/MS mass spectrometry analysis of unmodified AMG102, sample #2.



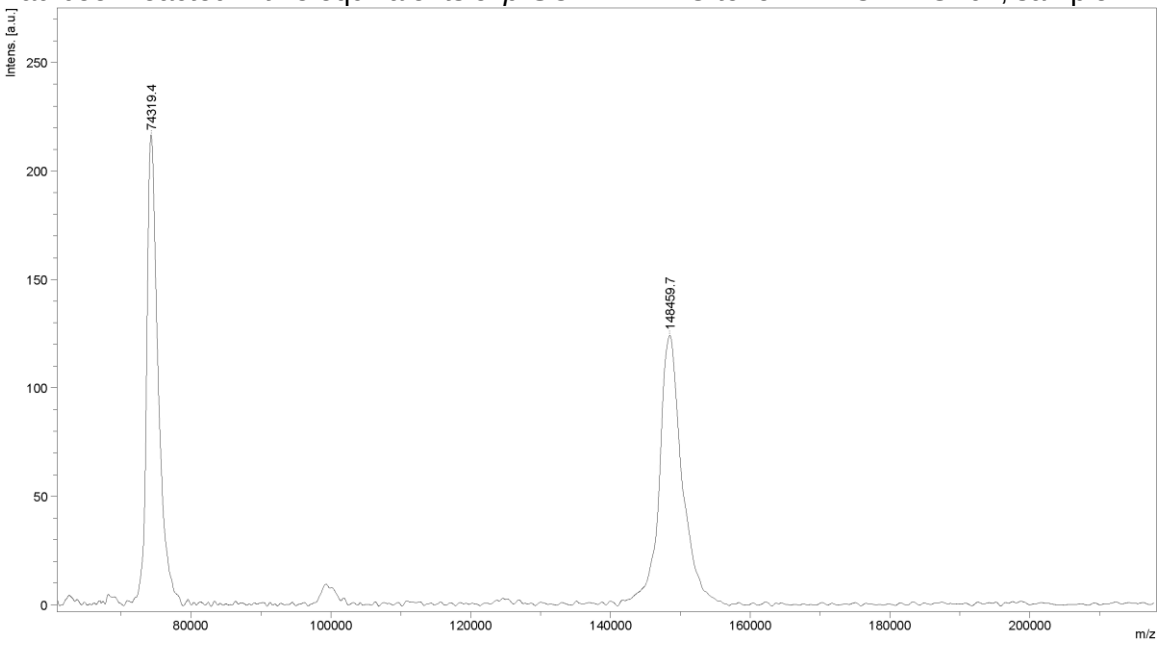

**Supplemental Fig. 3.** MALDI-TOF MS/MS mass spectrometry analysis of unmodified AMG102, sample #3.




**Supplemental Fig. 4.** MALDI-TOF MS/MS mass spectrometry analysis of AMG102 that had been reacted with 5 equivalents of *p*-SCN-Bn-DFO to form DFO-AMG102, sample #1.




**Supplemental Fig. 5.** MALDI-TOF MS/MS mass spectrometry analysis of AMG102 that had been reacted with 5 equivalents of *p*-SCN-Bn-DFO to form DFO-AMG102, sample #2.




**Supplemental Fig. 6.** MALDI-TOF MS/MS mass spectrometry analysis of AMG102 that had been reacted with 5 equivalents of *p*-SCN-Bn-DFO to form DFO-AMG102, sample #3.

**Supplemental Table 1**. MALDI-TOF MS/MS results of AMG102 and AMG102-DFO, submitted as 1 µg/µL solutions, 10 µL total, analyzed by the University of Alberta Mass Spectrometry Facility, with mass units as daltons (Da).

| | AMG102 standard (m+2/2) | Full mass | AMG102 DFO (m+2/2) | Full mass |
|---|---|---|---|---|
| **Mass (Da)** | 73900.8 | 147801.6 | 74225.7 | 148451.4 |
| **Mass (Da)** | 73913.2 | 147826.4 | 74303.0 | 148606.0 |
| **Mass (Da)** | 73884.2 | 147768.4 | 74319.4 | 148638.8 |
| **Average Mass (Da)** | 73899.4 | 147798.8 | 74282.7 | 148565.4 |
| **Std. Dev (Da)** | 14.6 | 29.1 | 50.0 | 100.1 |

| Immunoconjugate | Mass difference (Da) | Chelate mass (Da) | Chelates/antibody average | Standard Deviation |
|---|---|---|---|---|
| **AMG102-DFO** | 766.6 | 752.9 | 1.0 | 0.1 |

**Supplemental Table 2**. Biodistribution data of $^{89}$Zr-DFO-AMG102 or $^{89}$Zr-DFO-IgG (as indicated in table) (~20-30 µCi, 0.74-1.1 MBq, ~5 µg, in 200-250 µL of sterile saline; tumor volume ~ 100-150 mm$^3$) in female nude athymic mice bearing subcutaneous xenografts of U87MG or MKN45 (as indicated in table).

| $^{89}$Zr-DFO-antibody | AMG102 | | | AMG102 | | | AMG102 | | |
|---|---|---|---|---|---|---|---|---|---|
| Tumor xenograft | U87MG | | | U87MG | | | U87MG | | |
| Biodistribution time | 24 h | | | 48 h | | | 72 h | | |
| Organ | %ID/g | Std. Dev. | n | %ID/g | Std. Dev. | n | %ID/g | Std. Dev. | n |
| Blood | 23.3 | 1.2 | 5 | 22.8 | 1.9 | 5 | 20.5 | 2.1 | 5 |
| Tumor | 19.1 | 3.2 | 5 | 30.2 | 5.9 | 5 | 40.3 | 3.9 | 5 |
| Heart | 6.6 | 0.9 | 5 | 5.3 | 0.1 | 5 | 5.4 | 0.5 | 5 |
| Lungs | 9.3 | 2.0 | 5 | 10.4 | 1.4 | 5 | 11.2 | 1.5 | 5 |
| Liver | 6.1 | 1.0 | 5 | 5.7 | 1.1 | 5 | 6.7 | 0.3 | 5 |
| Spleen | 4.1 | 0.1 | 5 | 5.0 | 0.5 | 5 | 5.9 | 0.5 | 5 |
| Pancreas | 2.1 | 0.3 | 5 | 2.1 | 0.4 | 5 | 2.0 | 0.2 | 5 |
| Stomach | 1.6 | 0.4 | 5 | 1.7 | 0.6 | 5 | 1.2 | 0.5 | 5 |
| Small Intestine | 2.1 | 0.2 | 5 | 2.4 | 0.5 | 5 | 2.1 | 0.3 | 5 |
| Large Intestine | 1.2 | 0.3 | 5 | 1.3 | 0.3 | 5 | 1.0 | 0.2 | 5 |
| Kidney | 6.1 | 0.6 | 5 | 6.8 | 0.7 | 5 | 6.0 | 0.8 | 5 |
| Muscle | 1.8 | 0.2 | 5 | 1.5 | 0.2 | 5 | 1.4 | 0.2 | 5 |
| Bone | 4.4 | 0.3 | 5 | 4.4 | 0.7 | 5 | 6.8 | 0.9 | 5 |
| Skin | 6.9 | 1.4 | 5 | 5.0 | 0.6 | 5 | 7.7 | 0.3 | 5 |

**Supplemental Table 3**. Biodistribution data of $^{89}$Zr-DFO-AMG102 or $^{89}$Zr-DFO-IgG (as indicated in table) (~20-30 µCi, 0.74-1.1 MBq, ~5 µg, in 200-250 µL of sterile saline; tumor volume ~ 100-150 mm$^3$) in female nude athymic mice bearing subcutaneous xenografts of U87MG or MKN45 (as indicated in table).

| $^{89}$Zr-DFO-antibody | AMG102 | | | AMG102 + blocking (500 µg AMG102) | | | Human IgG | | | AMG102 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Tumor xenograft | U87MG | | | U87MG | | | U87MG | | | MKN45 | | |
| Biodistribution time | 120 h | | | 120 h | | | 120 h | | | 120 h | | |
| Organ | %ID/g | Std. Dev. | n | %ID/g | Std. Dev. | n | %ID/g | Std. Dev. | n | %ID/g | Std. Dev. | n |
| Blood | 15.2 | 2.0 | 5 | 19.4 | 1.4 | 5 | 9.9 | 4.3 | 5 | 10.6 | 1.9 | 10 |
| Tumor | 36.8 | 7.8 | 5 | 29.7 | 8.6 | 5 | 11.5 | 3.3 | 5 | 5.0 | 1.3 | 10 |
| Heart | 4.9 | 0.1 | 5 | 6.4 | 0.8 | 5 | 3.7 | 1.1 | 5 | 2.7 | 0.5 | 10 |
| Lungs | 8.3 | 1.8 | 5 | 10.4 | 1.6 | 5 | 5.7 | 1.8 | 5 | 5.9 | 1.0 | 10 |
| Liver | 5.6 | 0.5 | 5 | 5.8 | 0.4 | 5 | 12.1 | 1.8 | 5 | 5.3 | 1.2 | 10 |
| Spleen | 5.3 | 0.8 | 5 | 5.7 | 0.9 | 5 | 7.2 | 1.3 | 5 | 3.5 | 0.6 | 10 |
| Pancreas | 1.9 | 0.3 | 5 | 2.0 | 0.3 | 5 | 1.3 | 0.3 | 5 | 1.0 | 0.2 | 10 |
| Stomach | 1.4 | 0.2 | 5 | 1.7 | 0.4 | 5 | 1.2 | 0.2 | 5 | 0.8 | 0.3 | 10 |
| Small Intestine | 1.4 | 0.1 | 5 | 1.7 | 0.1 | 5 | 1.3 | 0.2 | 5 | 0.9 | 0.2 | 10 |
| Large Intestine | 0.8 | 0.1 | 5 | 0.8 | 0.1 | 5 | 0.6 | 0.1 | 5 | 0.7 | 0.2 | 10 |
| Kidney | 5.0 | 0.5 | 5 | 5.8 | 1.3 | 5 | 5.2 | 0.8 | 5 | 3.7 | 0.5 | 10 |
| Muscle | 1.3 | 0.2 | 5 | 1.5 | 0.3 | 5 | 0.9 | 0.2 | 5 | 0.7 | 0.1 | 10 |
| Bone | 6.2 | 1.0 | 5 | 7.0 | 2.4 | 5 | 10.0 | 2.4 | 5 | 2.1 | 0.3 | 10 |
| Skin | 5.4 | 1.2 | 5 | 5.9 | 0.5 | 5 | 4.4 | 0.3 | 5 | 4.8 | 1.1 | 10 |

**Supplemental Table 4**. Biodistribution data displayed as %ID of $^{89}$Zr-DFO-AMG102 (~20-30 µCi, 0.74-1.1 MBq, ~5 µg, in 200-250 µL of sterile saline; tumor volume ~ 100-150 mm$^3$) in female nude athymic mice bearing subcutaneous xenografts of U87MG, with data displayed as %ID to properly highlight blocking efficacy, due to dramatic tumor shrinkage observed in the blocking group.

| | U87MG, $^{89}$Zr-DFO-AMG102, 120 h | | | U87MG, $^{89}$Zr-DFO-AMG102, 500 µg block, 120 h | | |
|---|---|---|---|---|---|---|
| Organ | %ID | Std. Dev. | n | %ID | Std. Dev. | n |
| Blood | 7.7 | 2.1 | 5 | 10.6 | 3.1 | 5 |
| Tumor | 8.1 | 3.7 | 5 | 1.9 | 1.3 | 5 |
| Heart | 0.6 | 0.1 | 5 | 0.8 | 0.2 | 5 |
| Lungs | 1.6 | 0.5 | 5 | 2.0 | 0.2 | 5 |
| Liver | 5.2 | 0.5 | 5 | 6.0 | 0.8 | 5 |
| Spleen | 0.6 | 0.1 | 5 | 0.5 | 0.1 | 5 |
| Pancreas | 0.4 | 0.04 | 5 | 0.3 | 0.1 | 5 |
| Stomach | 0.5 | 0.1 | 5 | 0.6 | 0.1 | 5 |
| Small Intestine | 2.2 | 0.1 | 5 | 2.5 | 0.4 | 5 |
| Large Intestine | 1.3 | 0.2 | 5 | 1.3 | 0.3 | 5 |
| Kidney | 2.2 | 0.2 | 5 | 2.3 | 0.5 | 5 |
| Muscle | 0.1 | 0.02 | 5 | 0.2 | 0.03 | 5 |
| Bone | 0.3 | 0.1 | 5 | 0.3 | 0.2 | 5 |
| Skin | 1.1 | 0.2 | 5 | 1.2 | 0.1 | 5 |

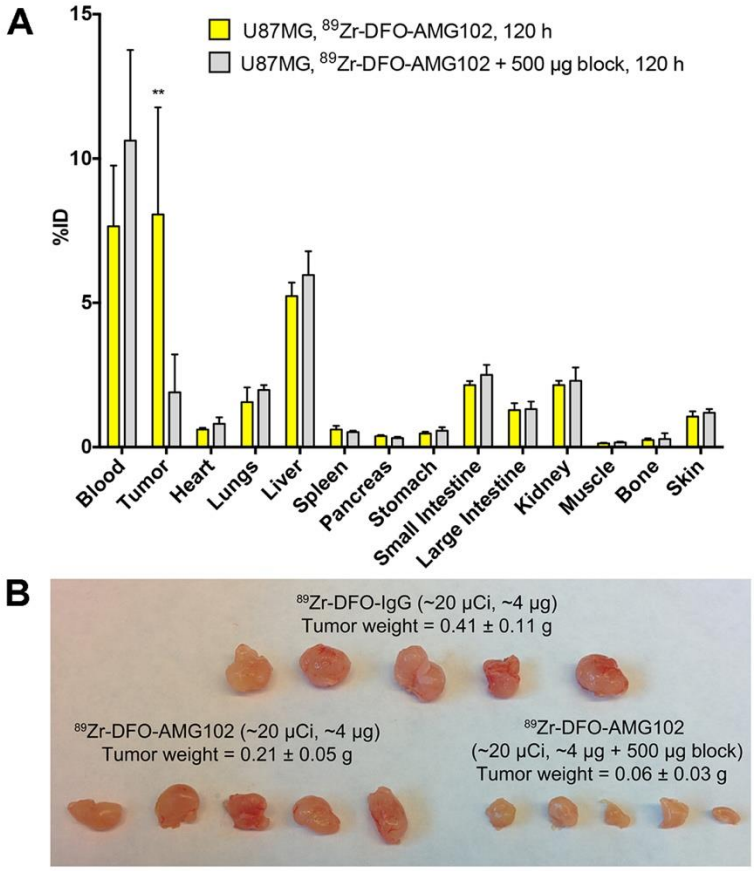
**Supplemental Table 5**. Biodistribution data shown as tumor/organ ratios of $^{89}$Zr-DFO-AMG102 or $^{89}$Zr-DFO-IgG (as indicated in table) (~20-30 µCi, 0.74-1.1 MBq, ~5 µg, in 200-250 µL of sterile saline; tumor volume ~ 100-150 mm$^3$) in female nude athymic mice bearing subcutaneous xenografts of U87MG or MKN45.

| $^{89}$ Zr-DFO-antibody | AMG102 | AMG102 | AMG102 | AMG102 | AMG102 block | human IgG | AMG102 |
|---|---|---|---|---|---|---|---|
| **Tumor xenograft** | **U87MG** | **U87MG** | **U87MG** | **U87MG** | **U87MG** | **U87MG** | **MKN45** |
| **Biodistribution time** | **24 h** | **48 h** | **72 h** | **120 h** | **120 h** | **120 h** | **120 h** |
| Tumor / Blood ratio | 0.8 | 1.3 | 2.0 | 2.4 | 1.5 | 1.2 | 0.4 |
| Tumor / Heart ratio | 2.9 | 5.7 | 7.5 | 7.5 | 4.6 | 3.1 | 1.7 |
| Tumor / Lungs ratio | 2.1 | 2.9 | 3.6 | 4.4 | 2.9 | 2.0 | 0.9 |
| Tumor / Liver ratio | 3.1 | 5.3 | 6.0 | 6.6 | 5.1 | 1.0 | 1.0 |
| Tumor / Kidney ratio | 3.1 | 4.4 | 6.8 | 7.3 | 5.1 | 2.2 | 1.4 |
| Tumor / Bone ratio | 4.4 | 6.9 | 5.9 | 6.0 | 4.3 | 1.2 | 2.5 |

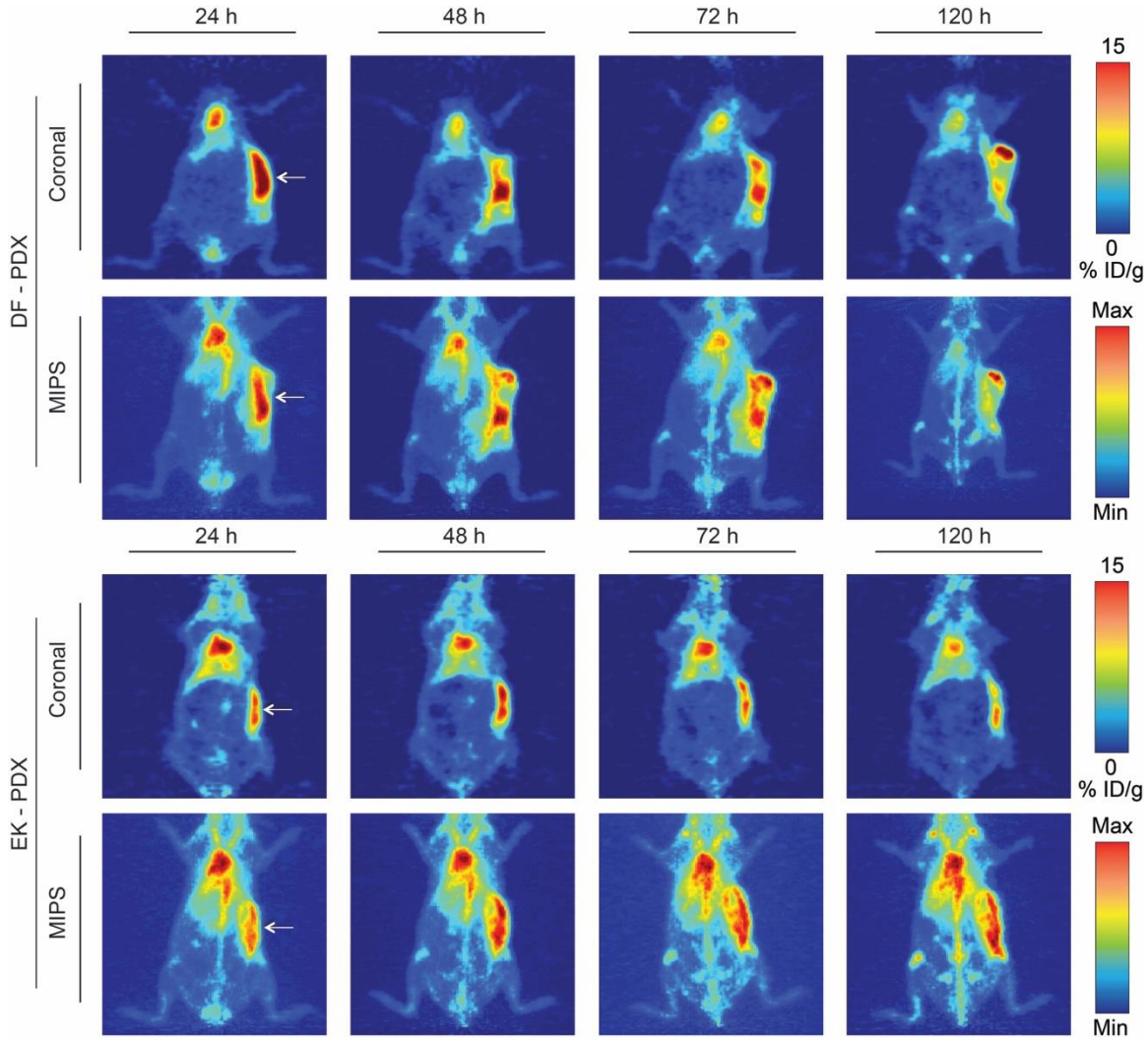
| **Tumor / organ Std. Dev.** | **24 h** | **48 h** | **72 h** | **120 h** | **120 h** | **120 h** | **120 h** |
|---|---|---|---|---|---|---|---|
| Tumor / Blood ratio | 0.1 | 0.3 | 0.3 | 0.6 | 0.5 | 0.6 | 0.1 |
| Tumor / Heart ratio | 0.6 | 1.1 | 1.0 | 1.6 | 1.5 | 1.3 | 0.5 |
| Tumor / Lungs ratio | 0.6 | 0.7 | 0.6 | 1.4 | 0.9 | 0.9 | 0.3 |
| Tumor / Liver ratio | 0.7 | 1.4 | 0.6 | 1.5 | 1.5 | 0.3 | 0.4 |
| Tumor / Kidney ratio | 0.6 | 1.0 | 1.1 | 1.7 | 1.9 | 0.7 | 0.4 |
| Tumor / Bone ratio | 0.8 | 1.7 | 0.9 | 1.6 | 1.9 | 0.4 | 0.7 |

**Supplemental Table 6**. Biodistribution data of $^{89}$Zr-DFO-AMG102 (~20-30 µCi, 0.74-1.1 MBq, ~5 µg, in 200-250 µL of sterile saline) post PET imaging at 120 h p.i. in patient-derived xenograft models (PDX) in female nude athymic mice bearing.

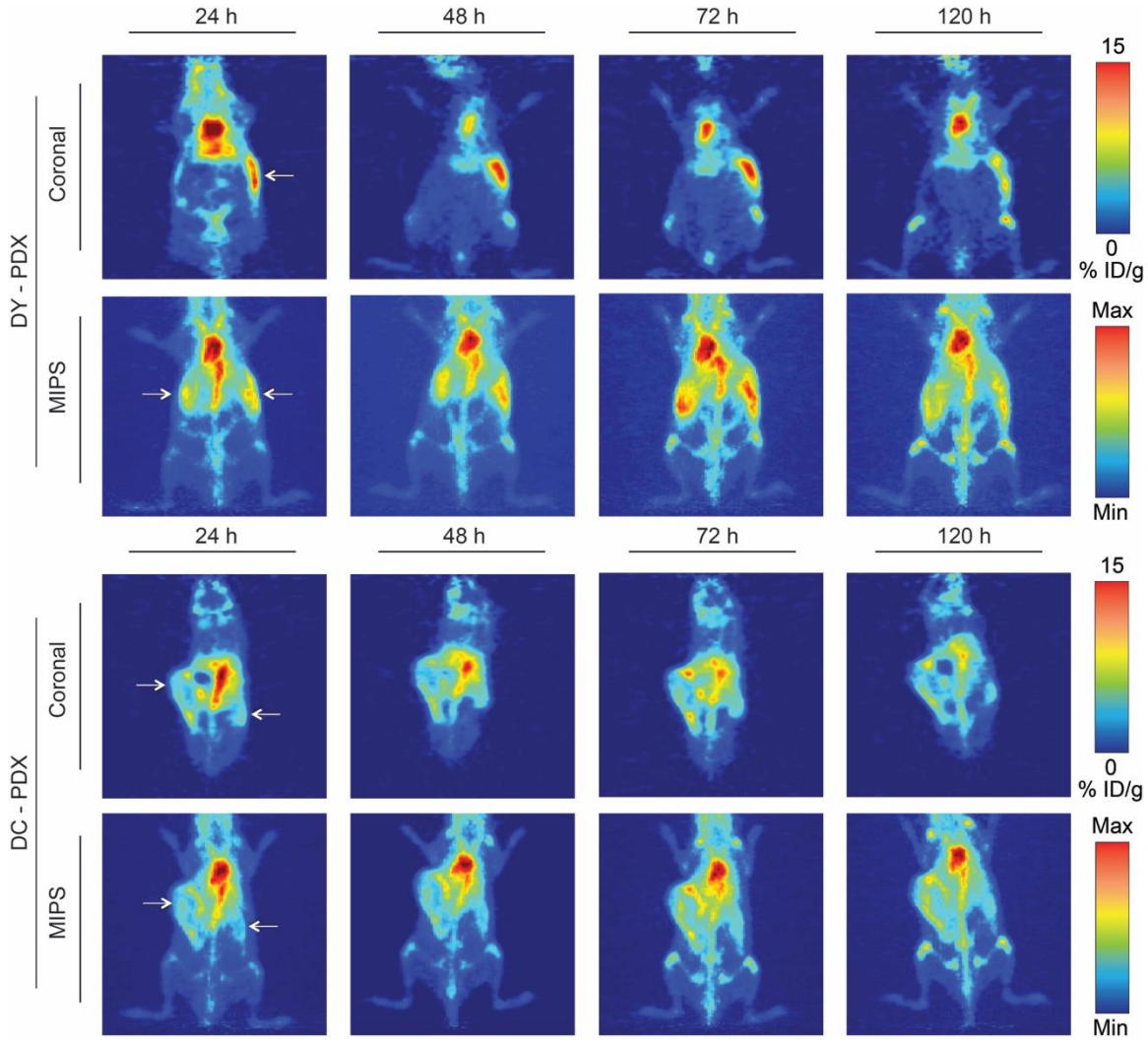
| Tumor xenograft | PDX-DY | | | PDX-DC | | | PDX-DF | | | PDX-EK | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Organ** | **%ID/g** | **Std. Dev.** | **n** | **%ID/g** | **Std. Dev.** | **n** | **%ID/g** | **Std. Dev.** | **n** | **%ID/g** | **Std. Dev.** | **n** |
| Blood | 15.1 | | 1 | 13.4 | | 1 | 11.6 | 4.6 | 2 | 13.4 | 2.3 | 3 |
| Tumor | 4.5 | 0.02 | 2 | 4.5 | 1.6 | 2 | 6.6 | 0.2 | 2 | 6.8 | 0.4 | 3 |
| Heart | 4.0 | | 1 | 3.0 | | 1 | 2.7 | 0.8 | 2 | 3.3 | 0.4 | 3 |
| Lungs | 9.3 | | 1 | 7.7 | | 1 | 6.2 | 3.2 | 2 | 5.8 | 1.4 | 3 |
| Liver | 5.3 | | 1 | 4.6 | | 1 | 4.7 | 0.3 | 2 | 6.4 | 0.7 | 3 |
| Spleen | 12.1 | | 1 | 11.4 | | 1 | 8.1 | 2.2 | 2 | 11.8 | 1.6 | 3 |
| Pancreas | 1.4 | | 1 | 1.3 | | 1 | 1.1 | 0.5 | 2 | 1.1 | 0.1 | 3 |
| Stomach | 1.5 | | 1 | 1.1 | | 1 | 1.0 | 0.03 | 2 | 0.8 | 0.2 | 3 |
| Small Intestine | 1.6 | | 1 | 1.5 | | 1 | 1.1 | 0.3 | 2 | 1.3 | 0.1 | 3 |
| Large Intestine | 1.1 | | 1 | 1.0 | | 1 | 0.8 | 0.1 | 2 | 0.9 | 0.1 | 3 |
| Kidney | 4.6 | | 1 | 4.6 | | 1 | 3.7 | 1.7 | 2 | 4.0 | 0.5 | 3 |
| Muscle | 1.2 | | 1 | 1.2 | | 1 | 0.8 | 0.3 | 2 | 0.7 | 0.1 | 3 |
| Bone | 5.6 | | 1 | 4.0 | | 1 | 4.7 | 2.6 | 2 | 3.9 | 0.5 | 3 |
| Skin | 5.4 | | 1 | 4.8 | | 1 | 3.4 | 1.1 | 2 | 5.8 | 1.4 | 3 |



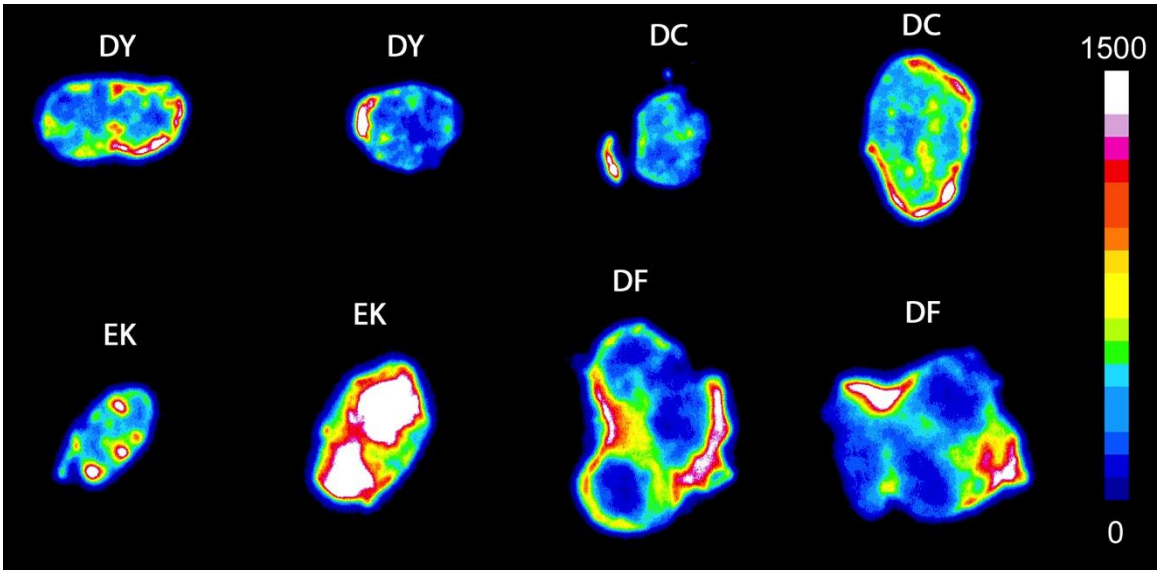

**Supplemental Fig. 7**. (A) a comparison of blocking and non-blocking groups from the biodistribution data of $^{89}$Zr-DFO-AMG102 at 120 with data expressed as %ID, showing similar %ID values for all tissues except for statistically significantly less uptake in blocked tumors; and (B) a photograph of tumors from these biodistribution experiments showing the difference in size between groups. Data in Supplemental Tables 2-5, statistical significance shown from students unpaired t-test using PRISM software, * = ≤ 0.05 ** = ≤ 0.01 *** = ≤ 0.001.



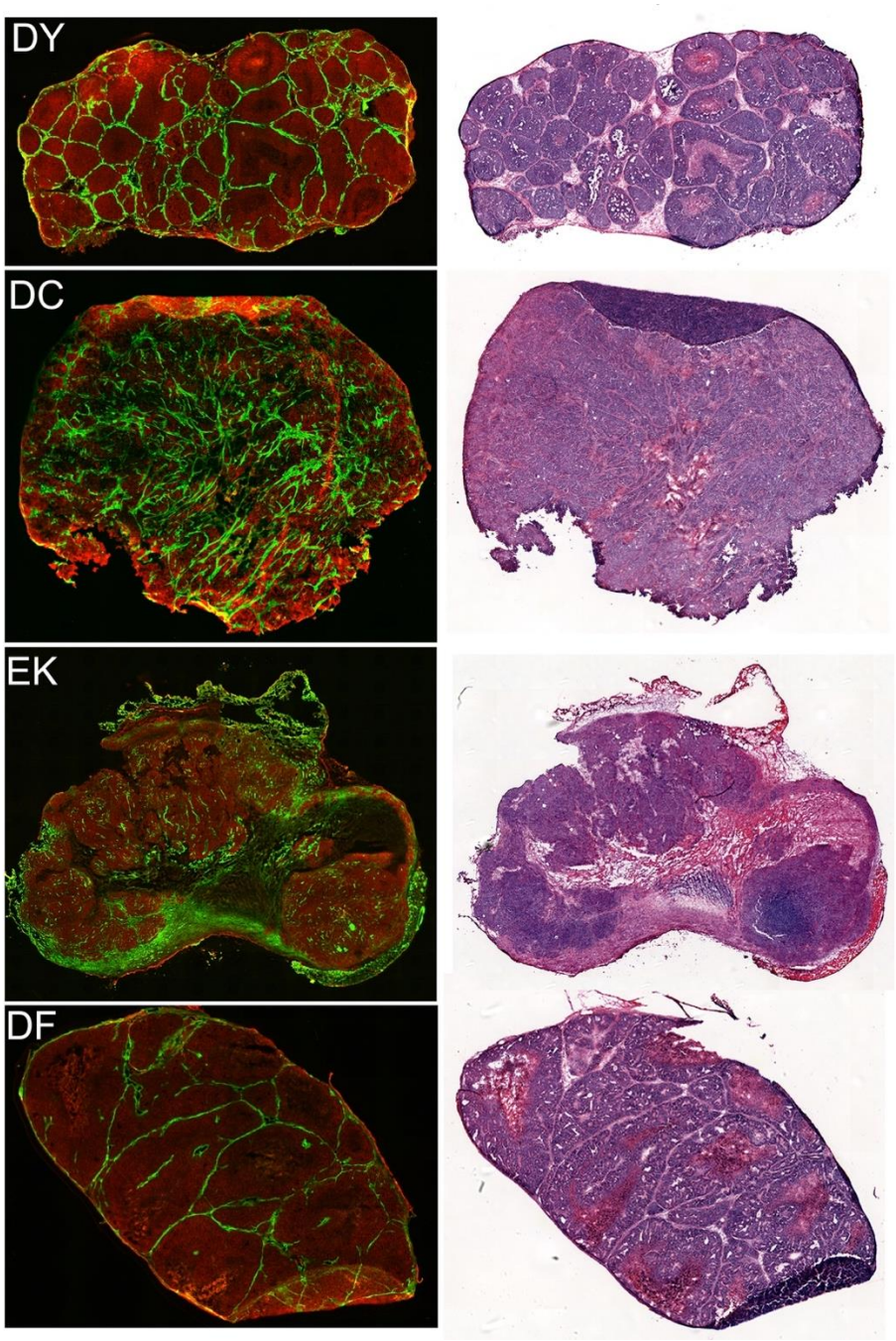

**Supplemental Fig. 8**. Serial PET imaging of $^{89}$Zr-DFO-AMG102 in PDX DF and EK bearing mice with unknown levels of HGF at 24, 48, 72, and 120 h p.i. (~30 µg, ~130-150 µCi, ~4.8-5.6 MBq, 200 µL sterile saline), showing low uptake in tumors suggesting very low levels of HGF protein, ~5-10 %ID/g, EPR uptake only.



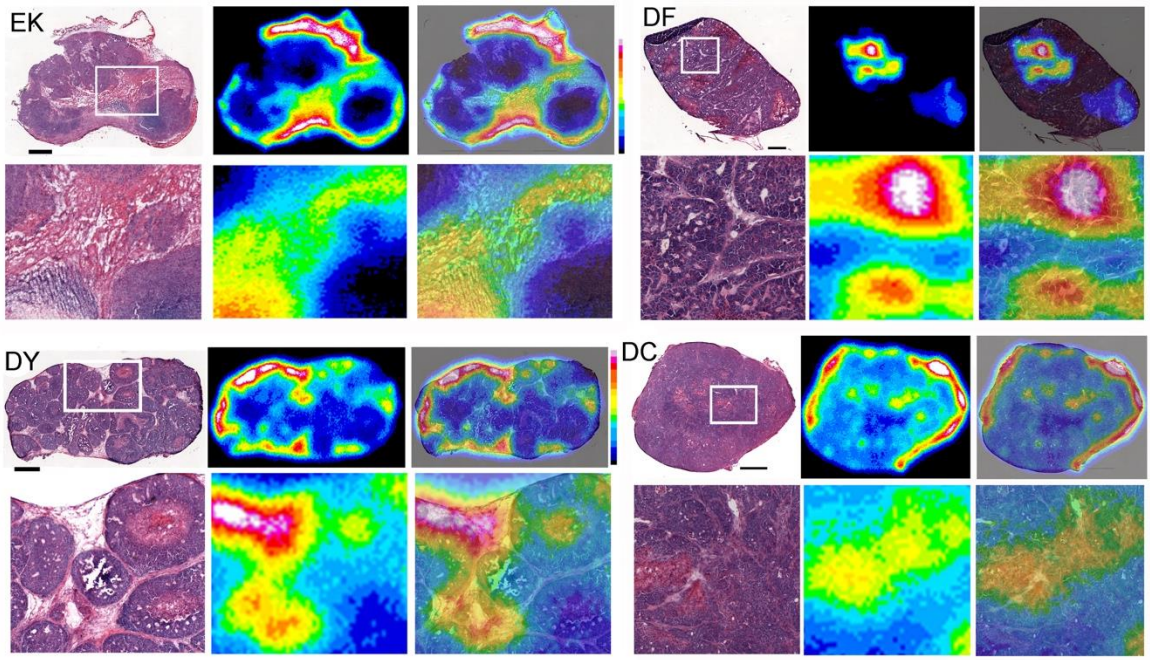

**Supplemental Fig. 9**. Serial PET imaging of $^{89}$Zr-DFO-AMG102 in PDX DF and EK bearing mice with unknown levels of HGF at 24, 48, 72, and 120 h p.i. (~30 µg, ~130-150 µCi, ~4.8-5.6 MBq, 200 µL sterile saline), showing low uptake in tumors suggesting very low levels of HGF protein, ~5-10 %ID/g, EPR uptake only.




**Supplemental Fig. 10**. Autoradiography of $^{89}$Zr-DFO-AMG102 in patient-derived xenograft mouse models (subcutaneous), obtained at time of necropsy (120 h p.i.) after PET imaging, with all tumor sections run on the same phosphor-plate to obtain relative radiation intensity.




**Supplemental Fig. 11**. Autoradiography of $^{89}$Zr-DFO-AMG102 in patient-derived xenograft mouse models (subcutaneous), obtained at time of necropsy (120 h p.i.) after PET imaging, with the images displayed showing histology from hematoxylin and eosin staining (right), and immunofluorescence staining shown in green for perlecan (extracellular matrix/stroma) and red for anti-HGF antibody on the left, with all 4 tumors having very little HGF present (red color intensity not normalized for image acquisition time, meaning the gain has been artificially turned up to visualize the red color, and despite the intense red color there is very little HGF actually present, as confirmed by ELISA assay). HGF levels in these samples are similar to those observed in MKN45 shown in the manuscript Fig. 7.




**Supplemental Fig. 12**. Autoradiography of $^{89}$Zr-DFO-AMG102 in patient-derived xenograft mouse models (subcutaneous), obtained at time of necropsy (120 h p.i.) after PET imaging, with the images displayed showing histology from hematoxylin and eosin staining (left), autoradiography (middle), and an overlap of both (right).