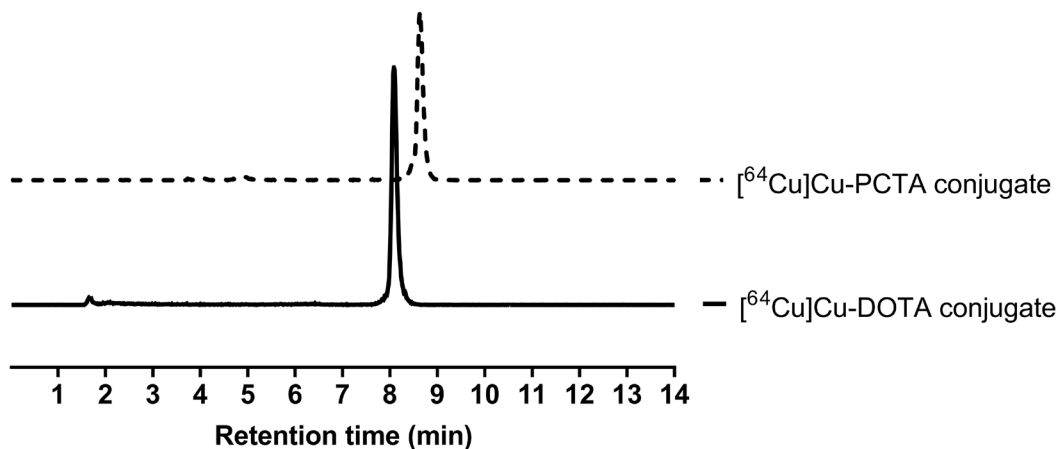
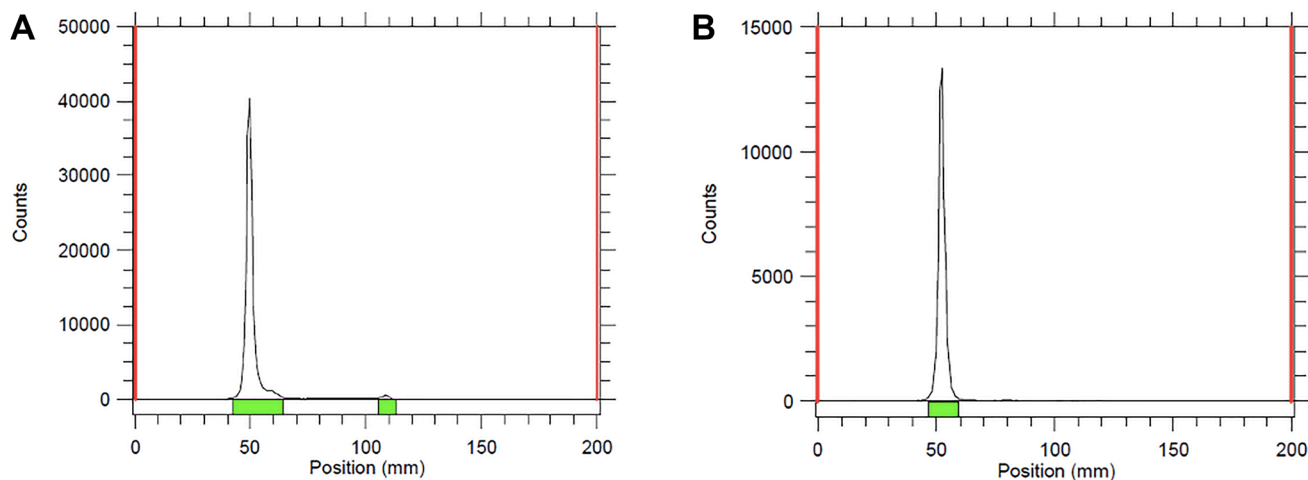


A comparison of ^{64}Cu -labeled bi-terminally PEGylated A20FMDV2 peptides targeting integrin $\alpha_v\beta_6$

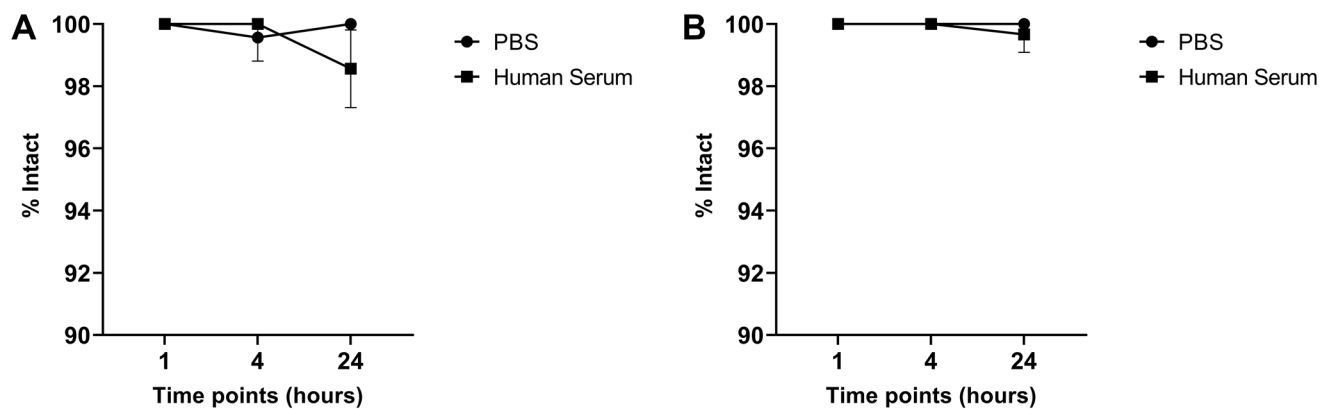
SUPPLEMENTARY MATERIALS



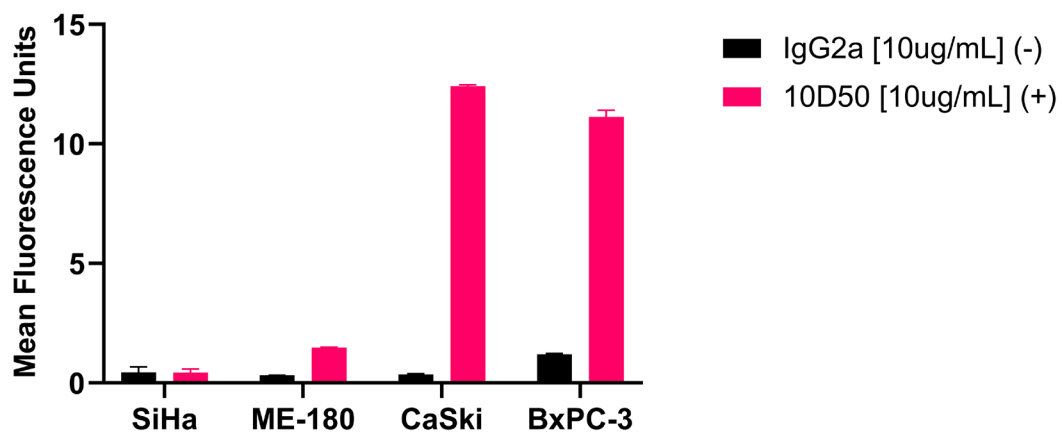
Supplementary Figure 1: HPLC chromatograms showing the ^{64}Cu labeled radiotracers. Radio-HPLC analysis was performed with a mobile phase of water (0.1% TFA) and acetonitrile (0.1% TFA), 20–100% acetonitrile from 0 to 14 mins, and elution was run with a 1 mL/min flow rate.



Supplementary Figure 2: Radio-TLC chromatograms of [^{64}Cu]Cu-DOTA-(PEG28)₂-A20FMDV2 (A) and [^{64}Cu]Cu-PCTA-(PEG28)₂-A20FMDV2 (B) in 50 mM DTPA.



Supplementary Figure 3: Stability profiles in PBS and human serum at 37°C up to 24 h of radiotracers (A) [⁶⁴Cu]Cu-DOTA-(PEG28)₂-A20FMDV2 and (B) [⁶⁴Cu]Cu-PCTA-(PEG28)₂-A20FMDV2.



Supplementary Figure 4: Expression of $\alpha_v\beta_6$ on a variety of cells lines as determined by flow cytometry. The bar graph shows mean fluorescence units (MFU) when cells were incubated with either the 10D5 antibody ($\alpha_v\beta_6$ -positive) or an IgG2a negative control isotype antibody.

Supplementary Table 1: Tumor-to-organ ratios of ^{64}Cu -labeled bi-terminally PEGylated A20FMDV2 peptides in $\alpha_v\beta_6$ -positive CaSki tumor-bearing mice

| | 1 h after injections | | | | 4 h after injection | | | | 24 h after injection | | | |
|---------------|----------------------|------|-------|-------|---------------------|-------|-------|------|----------------------|------|-------|------|
| | DOTA | | PCTA | | DOTA | | PCTA | | DOTA | | PCTA | |
| | Ratio | SD | Ratio | SD | Ratio | SD | Ratio | SD | Ratio | SD | Ratio | SD |
| Blood | 4.87 | 1.48 | 2.98 | 0.29 | 4.80 | 0.94 | 3.09 | 1.83 | 2.63 | 1.07 | 2.96 | 0.99 |
| Lung | 0.71 | 0.21 | 0.44 | 0.15 | 0.52 | 0.13 | 0.61 | 0.32 | 0.66 | 0.18 | 0.78 | 0.40 |
| Liver | 0.90 | 0.18 | 0.24 | 0.06 | 0.45 | 0.11 | 0.34 | 0.30 | 0.48 | 0.17 | 0.44 | 0.22 |
| Spleen | 5.81 | 0.77 | 2.56 | 0.76 | 3.11 | 0.47 | 2.28 | 1.39 | 1.72 | 0.51 | 1.70 | 0.49 |
| Muscle | 1.36 | 0.30 | 1.33 | 0.08 | 1.71 | 0.19 | 2.57 | 2.15 | 2.66 | 1.03 | 2.59 | 0.86 |
| Heart | 1.37 | 0.34 | 0.65 | 0.06 | 1.48 | 0.09 | 1.53 | 1.25 | 1.30 | 0.45 | 1.21 | 0.75 |
| Bone | 3.36 | 2.59 | 3.20 | 1.09 | 5.51 | 189 | 5.22 | 2.88 | 5.89 | 3.17 | 3.02 | 1.22 |
| Kidney | 0.02 | 0.01 | 0.01 | 0.001 | 0.01 | 0.002 | 0.02 | 0.01 | 0.04 | 0.01 | 0.07 | 0.05 |

Supplementary Table 2: Tumor-to-organ ratios of ^{64}Cu -labeled bi-terminally PEGylated A20FMDV2 peptides in $\alpha_v\beta_6$ -positive BxPC-3 tumor-bearing mice

| | 1 h after injections | | | | 4 h after injection | | | | 24 h after injection | | | |
|---------------|----------------------|------|-------|------|---------------------|------|-------|------|----------------------|------|-------|------|
| | DOTA | | PCTA | | DOTA | | PCTA | | DOTA | | PCTA | |
| | Ratio | SD | Ratio | SD | Ratio | SD | Ratio | SD | Ratio | SD | Ratio | SD |
| Blood | 7.86 | 2.21 | 14.25 | 0.86 | 5.53 | 1.23 | 13.65 | 2.44 | 5.49 | 1.37 | 9.07 | 4.49 |
| Lung | 1.21 | 0.40 | 1.51 | 0.21 | 1.12 | 0.14 | 1.71 | 0.27 | 1.31 | 0.19 | 1.44 | 0.45 |
| Liver | 0.76 | 0.23 | 2.02 | 0.44 | 0.63 | 0.11 | 1.29 | 0.05 | 0.64 | 0.07 | 1.07 | 0.28 |
| Spleen | 7.06 | 2.08 | 14.36 | 3.70 | 4.21 | 0.92 | 11.45 | 3.39 | 2.93 | 0.28 | 7.60 | 4.24 |
| Muscle | 3.37 | 0.68 | 3.51 | 0.78 | 4.60 | 0.43 | 3.75 | 0.18 | 5.38 | 1.12 | 4.84 | 0.60 |
| Heart | 2.06 | 0.57 | 2.04 | 0.32 | 2.62 | 0.21 | 2.72 | 0.25 | 2.14 | 0.34 | 2.67 | 0.75 |
| Bone | 9.29 | 2.53 | 10.94 | 6.20 | 8.31 | 1.67 | 19.22 | 9.75 | 7.71 | 2.66 | 10.97 | 5.97 |
| Kidney | 0.06 | 0.03 | 0.04 | 0.01 | 0.05 | 0.01 | 0.03 | 0.01 | 0.09 | 0.02 | 0.10 | 0.07 |

Supplementary Table 3: Biodistribution profile of ^{64}Cu -labeled bi-terminally PEGylated A20FMDV2 peptides in $\alpha_v\beta_6$ -positive CaSki tumor-bearing mice (1 h and 1 h Block)

| | DOTA | | | | PCTA | | | |
|---------------|-------|------|-----------|------|--------|-------|-----------|------|
| | 1 h | | 1 h Block | | 1 h | | 1 h Block | |
| | %ID/g | SD | %ID/g | SD | %ID/g | SD | %ID/g | SD |
| Blood | 0.22 | 0.14 | 0.13 | 0.02 | 0.54 | 0.15 | 0.76 | 0.30 |
| Lung | 1.37 | 0.36 | 1.19 | 0.22 | 3.76 | 0.66 | 1.43 | 0.26 |
| Liver | 1.04 | 0.14 | 0.94 | 0.16 | 6.81 | 1.67 | 3.04 | 1.32 |
| Spleen | 0.17 | 0.06 | 0.13 | 0.02 | 0.63 | 0.05 | 0.54 | 0.14 |
| Muscle | 0.73 | 0.33 | 0.51 | 0.10 | 1.23 | 0.04 | 0.43 | 0.25 |
| Heart | 0.71 | 0.24 | 0.58 | 0.04 | 2.46 | 0.58 | 0.55 | 0.07 |
| Bone | 0.62 | 0.76 | 0.27 | 0.19 | 0.54 | 0.15 | 0.39 | 0.08 |
| Kidney | 42.72 | 4.03 | 35.48 | 4.38 | 113.58 | 38.31 | 43.56 | 5.63 |
| Tumor | 0.95 | 0.29 | 0.65 | 0.17 | 1.63 | 0.53 | 0.67 | 0.26 |

Supplementary Table 4: Biodistribution profile of ⁶⁴Cu-labeled bi-terminally PEGylated A20FMDV2 peptides in $\alpha_v\beta_6$ -positive BxPC-3 tumor-bearing mice (1 h and 1 h Block)

| | DOTA | | | | PCTA | | | |
|---------------|-------|------|-----------|------|--------|-------|-----------|-------|
| | 1 h | | 1 h Block | | 1 h | | 1 h Block | |
| | %ID/g | SD | %ID/g | SD | %ID/g | SD | %ID/g | SD |
| Blood | 0.28 | 0.07 | 0.24 | 0.07 | 0.27 | 0.03 | 0.27 | 0.11 |
| Lung | 1.73 | 0.29 | 1.14 | 0.20 | 2.58 | 0.41 | 1.25 | 0.27 |
| Liver | 2.79 | 0.59 | 2.59 | 0.36 | 1.94 | 0.20 | 1.68 | 0.20 |
| Spleen | 0.30 | 0.06 | 0.34 | 0.10 | 0.28 | 0.04 | 0.24 | 0.05 |
| Muscle | 0.62 | 0.22 | 0.18 | 0.06 | 1.13 | 0.24 | 0.28 | 0.12 |
| Heart | 1.01 | 0.15 | 0.34 | 0.06 | 1.90 | 0.25 | 0.27 | 0.02 |
| Bone | 0.25 | 0.12 | 0.28 | 0.14 | 0.38 | 0.31 | 0.66 | 0.92 |
| Kidney | 38.04 | 5.47 | 27.60 | 2.79 | 105.39 | 13.58 | 80.64 | 15.50 |
| Tumor | 2.13 | 0.83 | 0.71 | 0.16 | 3.86 | 0.58 | 0.75 | 0.09 |