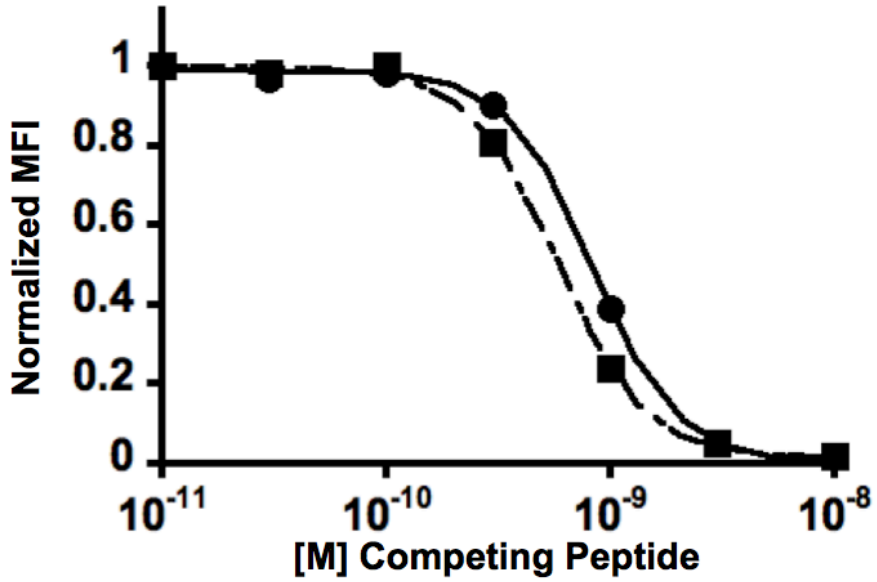
SUPPLEMENTAL SECTION

A Radiofluorinated Divalent Cystine Knot Peptide for Tumor PET Imaging

Lei Jiang,[†,‡,§] Richard H. Kimura,[‡,§] Xiaowei Ma,[‡] Yingfeng Tu,[‡] Zheng Miao,[‡] Bin Shen,[‡] Frederick T. Chin,[‡] Hongcheng Shi,[†] Sanjiv Sam Gambhir,[‡] and Zhen Cheng*[,‡]

[†]Department of Nuclear Medicine, Zhongshan Hospital, Fudan University, 180 Fenglin Road, Shanghai, China 200032

[‡]Molecular Imaging Program at Stanford (MIPS), Department of Radiology and Bio-X Program, Canary Center at Stanford for Cancer Early Detection, Stanford University, Stanford, California 94305, United States



SUPPLEMENTAL FIGURE 1. Binding Affinity of 3-4A and ^{19}F-FP-3-4A. The IC_{50} of ^{19}F-FP-3-4A was compared that of to 3-4A. These competing peptides were incubated with yeast (surface) displaying 3-4A c-myc fusion protein, and allowed to compete with the soluble peptides for 20nM integrin $\alpha_v\beta_3$. The IC_{50} of soluble 3-4A and ^{19}F-FP-3-4A was 615.6 +/- 305.8 pM and 810.4+/- 343.6pM, respectively

SUPPLEMENTAL METHOD 1. Saccharomyces Cerevisiae cells were transformed with plasmid, pCT, encoding dual RGD knottin 3-4A. Cells were grown in SD-CAA media and proteins were induced in SG-CAA (1). For each time point, 100,000 cells expressing 3-4A was incubated with 20nM integrin avb3 (www.rndsystems.com), and several serially-diluted concentrations of peptides 3-4A and 19F-FP-3-4A to generate titration curves. Yeast surface expression of fusion proteins was measured with a chicken anti-c-myc IgY antibody (A-21281, www.invitrogen.com), and Alexa-555 labeled goat anti-chicken IgG antibody (A-21437, www.invitrogen.com). Integrin avb3 binding was measured with FITC-labeled anti integrin av antibody (Clone NKI-M9, www.biolegend.com).

Mean Fluorescence Intensity (MFI) is defined by FITC signal intensity divided by Alexa 555 signal intensity. MFI values (Y-axis) were determined for each concentration of competing peptide (X-axis). Kaleidagraph (www.synergy.com) was used to determine the IC_{50} values.

REFERENCES

1. Kimura, R. H.; Jones, D. S.; Jiang, L.; Miao, Z.; Cheng, Z.; Cochran, J. R. Functional mutation of multiple solvent-exposed loops in the Ecballium elaterium trypsin inhibitor-II cystine knot miniprotein. *PLoS One* **2011,** *6*, (2), e16112.