

Supporting Information

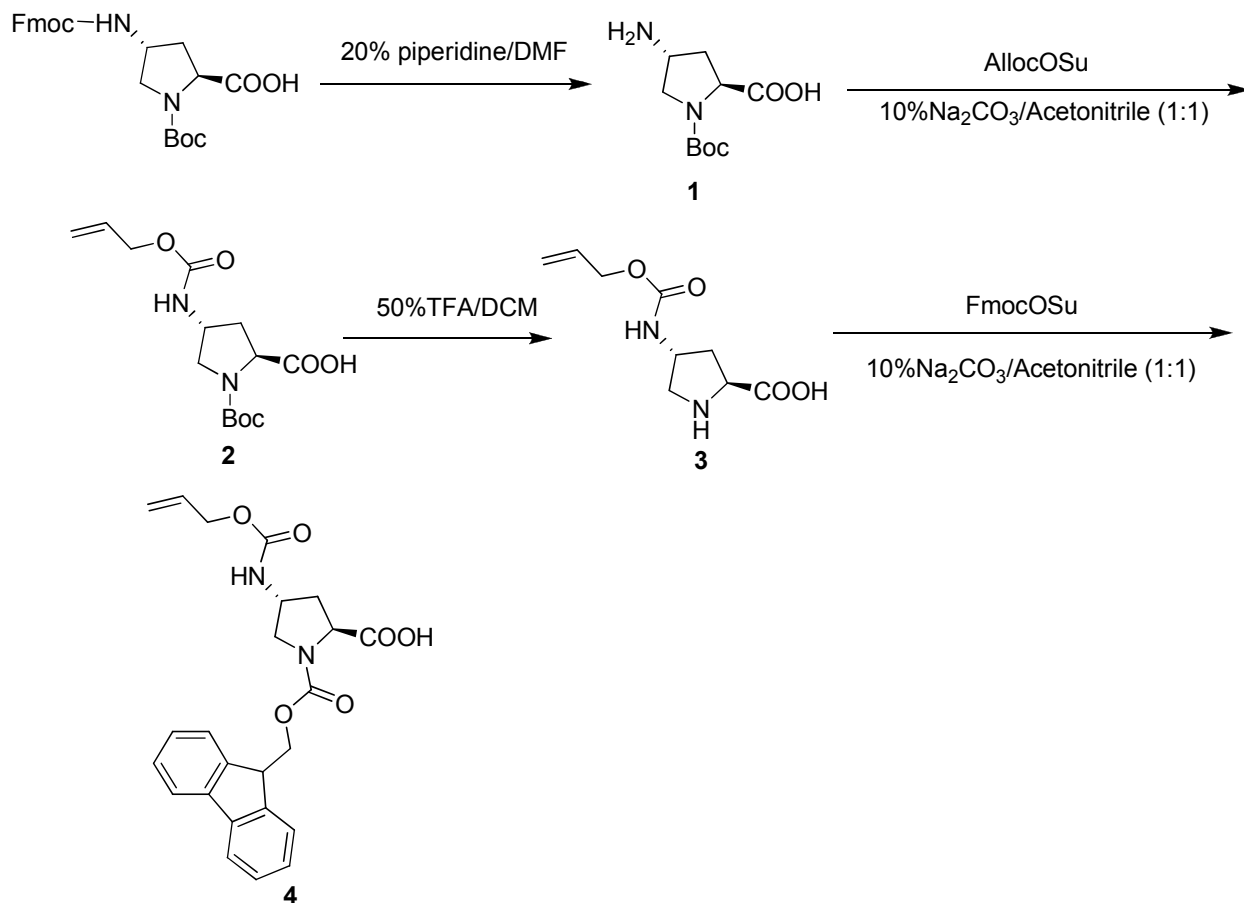
Structure –Activity Relationship Studies for Targeting Ligands against Breast Cancer Cells

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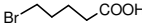
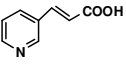
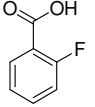
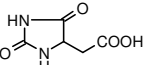
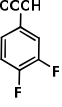
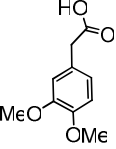
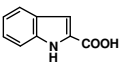
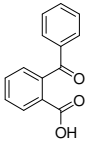
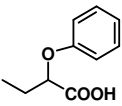
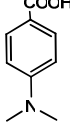
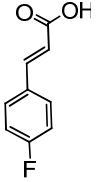
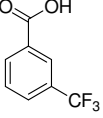
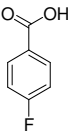
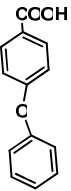
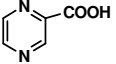
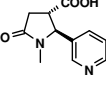
Scheme S1. Synthetic route of (2S, 4R)-Alloc-4-amino-1-Fmoc-pyrrolidine-2-carboxylic acid

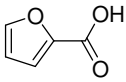
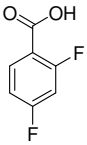
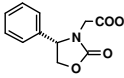
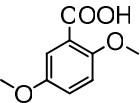
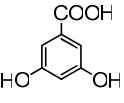
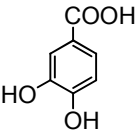
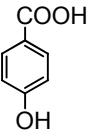
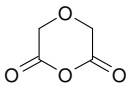
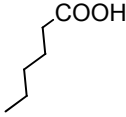
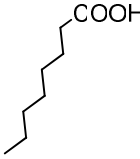
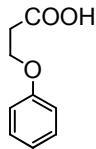

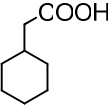
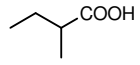
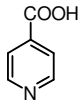
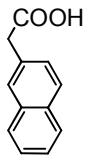
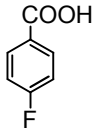
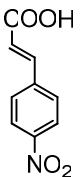
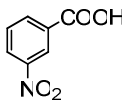
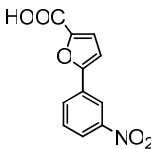
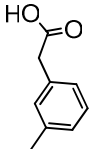
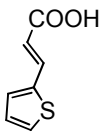


(2S, 4R)-Fmoc-4-amino-1-Boc-pyrrolidine-2-carboxylic acid (**1g**) was added to 20% 2mL piperidine /DMF, and stirred for 15min. To the mixture, 50mL cold diethyl ether was added to precipitate the product (**1**, 524mg). **1**(460mg) was dissolved in 10% (w/v) aqueous solutions of sodium carbonate (5mL). A solution of AllocOSu(398mg) in acetonitrile(5mL) was then added dropwise. The mixture was stirred overnight at room temperature, and acidified with 6N HCl to PH 1. The solution was extracted with ethyl acetate three times. Ethyl acetate was collected and concentrated to give **2**. **2** was treated with 50%TFA/DCM (10mL) for 50min at room temperature, and dried with air flow. Following precipitation with cold diethyl ether, the solid (**3**) was dissolved in 10% (w/v) aqueous solutions of sodium carbonate (5mL). A solution of FmocOSu(236mg) in acetonitrile(5mL) was then added dropwise. The mixture was stirred

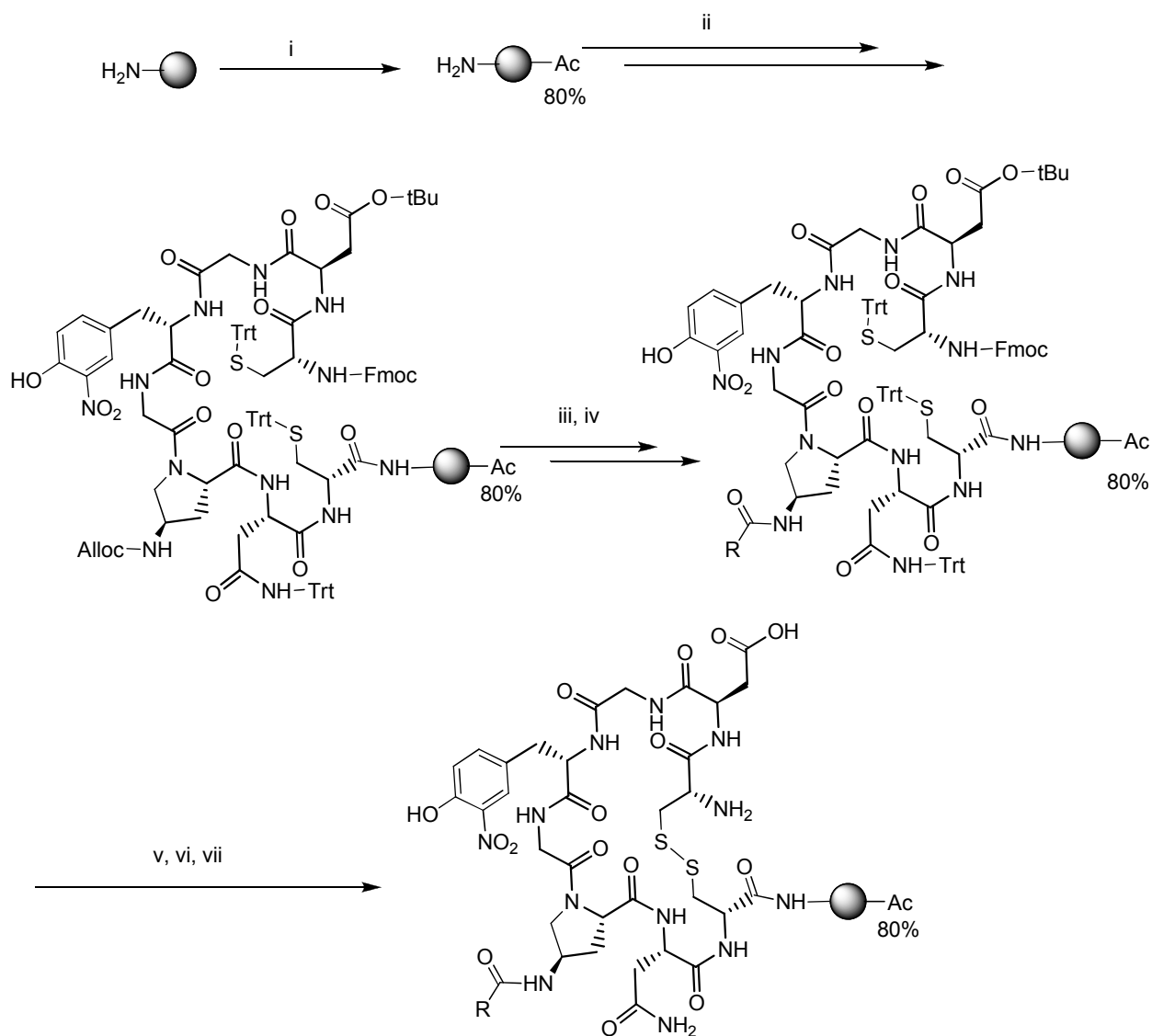
overnight at room temperature, and neutralized to PH1. The solution was extracted with ethyl acetate three times. Ethyl acetate was collected and purified to give the final product (4). ^1H NMR(DMSO- d_6 , 500MHz): δ ppm 7.90(2H, t, $J=5\text{Hz}$), 7.65(3H, m), 7.42(2H, t, $J=5\text{Hz}$), 7.33(2H, t, $J=5\text{Hz}$), 5.92(1H, m), 5.3(1H, brd), 5.19(1H, brd), 4.48(2H, m), 4.30(1H, m), 4.27(2H, brs), 4.16(2H, m), 3.61(1H, m), 3.35(1H, m), 2.18(1H, m), 2.08(1H, m). ESI-MS m/z : calcd for $\text{C}_{24}\text{H}_{24}\text{N}_2\text{O}_6$ $[\text{M}+1]^+$, 437.1, found 437.2. Purity 95%

Table S1. The structure of carboxylic acids

<p>1</p> <p>5-Bromovaleric acid</p> 	<p>2</p> <p>trans-3-(3-Pyridyl)acrylic acid</p> 	<p>3</p> <p>2-Fluorobenzoic acid</p> 	<p>4</p> <p>5-Hydantoinacetic acid</p> 	<p>5</p> <p>,4-Difluorobenzoic acid</p> 	<p>6</p> <p>(3,4-Dimethoxyphenyl)acetic acid</p> 	<p>7</p> <p>Indole-2-carboxylic acid</p> 	<p>8</p> <p>2-Benzoylbenzoic acid</p> 
<p>9</p> <p>2-Phenoxybutyric acid</p> 	<p>10</p> <p>4-(Dimethylamino)benzoic acid</p> 	<p>11</p> <p>4-Fluorocinnamic acid</p> 	<p>12</p> <p>α,α,α-Trifluorom-toluic acid</p> 	<p>13</p> <p>4-Fluorobenzoic acid</p> 	<p>14</p> <p>4-Phenoxybenzoic acid</p> 	<p>15</p> <p>2-Pyrazinecarboxylic acid</p> 	<p>16</p> <p>Trans-4-Cotininecarboxylic acid</p> 

17 2-Furoic acid 	18 2,4-Difluorobenzoic acid 	19 (S)-(+)-Oxo-4-phenyl-3-oxazolidinacetic acid 	20 2,5-Dimethoxyphenyl acetic acid 	21 3,5-Dihydroxybenzoic acid 	22 3,4-Dihydroxybenzoic acid 	23 4-Hydroxybenzoic acid 	24 Diglycolic anhydride 
25 Valeric acid 	26 Octanoic acid 	27 3-Phenoxypropionic acid 	28 Cyclohexanecarboxylic acid 	29 Cyclohexylacetic acid 	30 2-Methylbutyric acid 	31 Isonicotinic acid 	32 2-Naphthylacetic acid 
33 4-Fluorobenzoic acid 	34 4-Nitrocinnamic acid 	35 3-Nitrobenzoic acid 	36 5-(3-Nitrophenyl)-2-furoic acid 	37 m-Tolylacetic acid 	38 Trnas-3-(3-Thienyl)acrylic acid 	39 NH ₂	

Scheme S2. Synthetic route of **1** analogues with Hyp modification

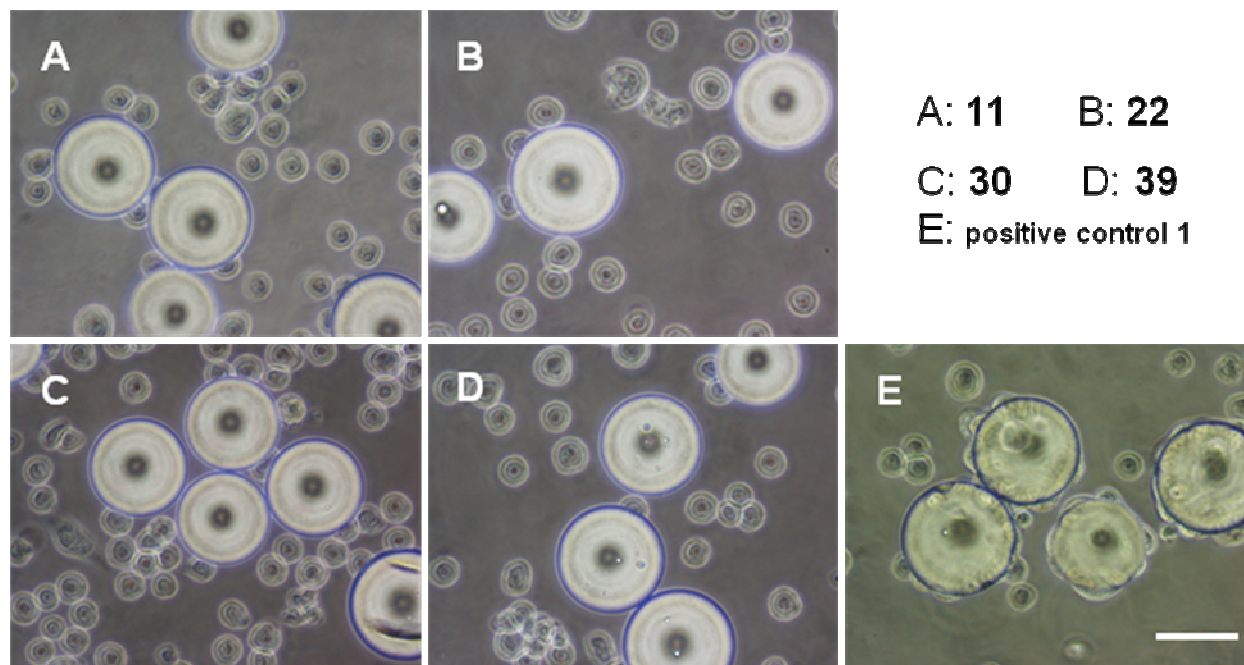


Reagents and conditions:

(i) [Fmoc-D-Cys(Trt)-OH (20%) and Ac-Gly-OH (80%)] /HOBt/DIC; (ii) Fmoc-aa-OH/HOBt/DIC(3equiv); (iii)Pd(Ph₃P)₄/PhSiH₃/DCM, 30min, rt, twice; (iv) R-COOH/HOBt/DIC(3equiv); (v), 20% piperidine/DMF; (vi) 82.5% TFA: 5% phenol: 5% thioanisole: 5% water: 2.5% triisopropylsilane(TIS); (vii) DMSO:H₂O:Acetic acid(20:75:5).

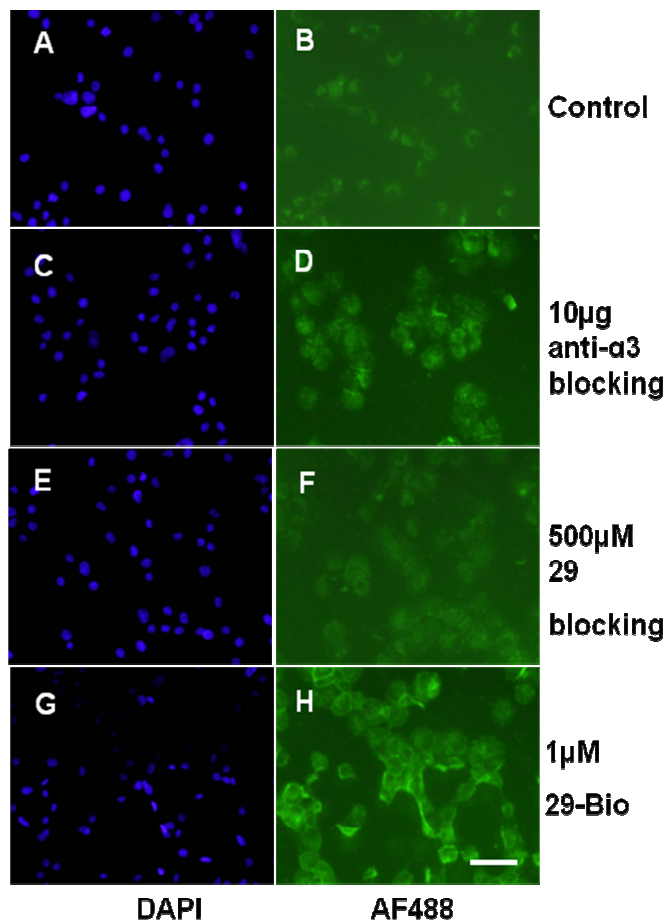
TentaGel S NH₂ beads (2.0g, loading 0.26mmol/g) were swollen in DMF (30mL) in a fritted column for 3h. After draining the solvent, 2 molar equivalents of mixture [Fmoc-D-Cys(Trt)-OH (20%) and Ac-Gly-OH (80%)] and 1-hydroxybenzotriazole (HOBt) in DMF, as well as DIC (3 equiv) were added to the beads to download the beads substitute to 20%. The column was agitated by rotation until a Kaiser test was negative. The resin was washed and subjected to Fmoc deprotection with 20% piperidine in DMF (5min, 15min). After washing with DMF, MeOH, DCM, and DMF, Fmoc-Asn(Trt)-OH, (2S, 4R)-Alloc-4-amino-1-Fmoc-pyrrolidine-2-carboxylic acid, Fmoc-Gly-OH, Fmoc-Tyr(3-NO₂)-OH, Fmoc-Gly-OH, Fmoc-D-Asp(OtBu)-OH, and Fmoc-D-Cys(Trt)-OH was sequentially assembled to the beads using Fmoc solid-phase chemistry. Following Alloc deprotection with Pd(Ph₃)₄ (0.24 equiv) and PhSiH₃ (20 equiv) in DCM for 30min, twice, the beads were split into 40 equal portions in 40 tubes (5mL). 38 different carboxylic (3equiv) (**Table 1**), HOBt (3 equiv) and DIC (3 equiv) were dissolved in DMF, and separately added to the 38 tubes. The coupling was carried out at room temperature for 2h. Four random tubes were chosen for a Kaiser test, and when it was negative, the beads drained and washed with DMF, three times. After Fmoc deprotection, the beads were sequentially washed three times each with DMF, Methanol, and DCM, and finally dried under vacuum. Side-chain deprotection was achieved using a mixture of 82.5% TFA: 5% phenol: 5% thioanisole: 5% water: 2.5% triisopropylsilane(TIS). After neutralization with 3% DIEA/DMF (twice), the beads was washed sequentially with DMF, MeOH, DCM, DMF, DMF/water (60%, 30%) and water. The beads were transferred to 10ml tubes respectively, to which was added 9mL mixture of water, acetic acid and DMSO (75:5:20). Tubes were shaken for two days until the Ellman test was negative. After filtration, the beads were thoroughly washed with H₂O. Finally, the bead library was stored in 75% ethanol/water and was ready for screening. The positive **1** beads were synthesized using the same strategy as above.

Figure S1. Examples for **1** analogues beads with Hyp modification binding to MDA-MB-231 breast cancer cells



LXY3 beads as positive control beads and analogues beads were incubated with MDA-MB-231 cells. Within 2 hrs, strong cell binding was shown on LX3 beads (E). No cell binding were observed on the analogue beads (A, B, C and D). Scale bar =100 μ m.

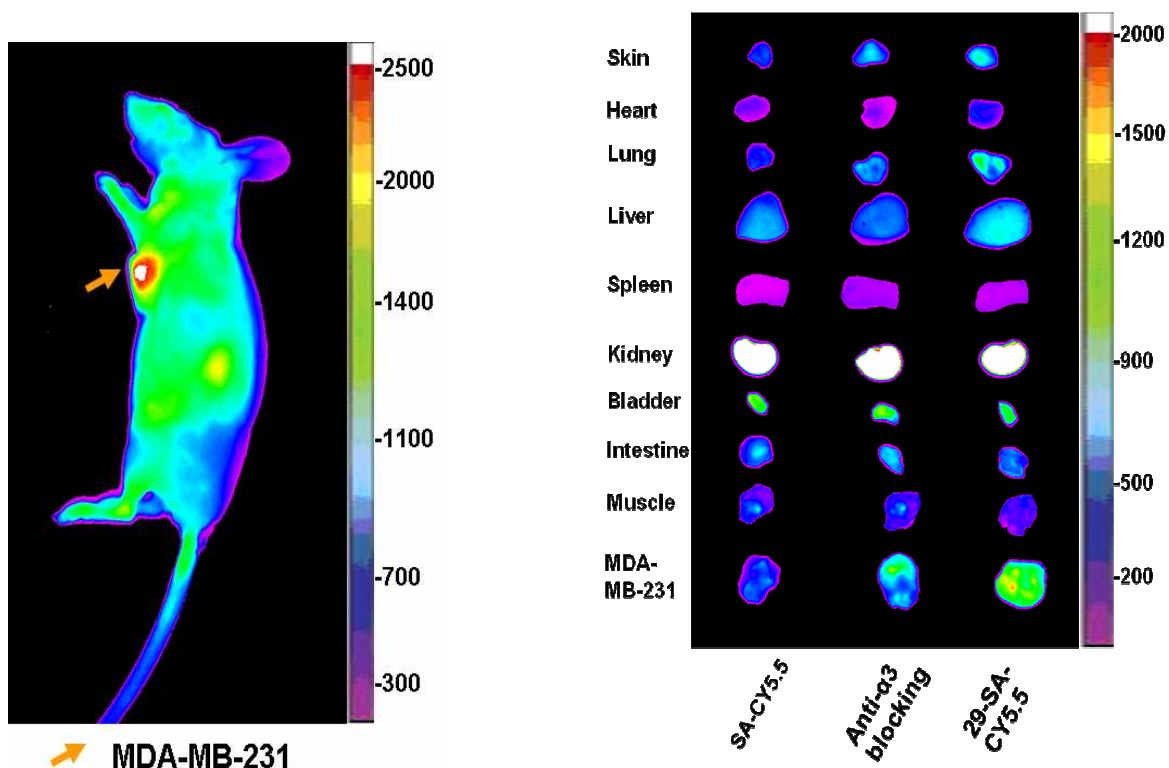
Figure S2. Fluorescent microscopy of MDA-MB-231 cells stained with biotinylated **29**



80-90% confluent MDA-MB-231 cells were dissociated with 0.05% trypsin-EDTA and neutralized with culture medium. 3×10^5 cells in each sample were incubated with biotinylated **29** in 50 μ L PBS containing 10% FBS and 1 mM MnCl_2 for 30 minutes on ice. After peptide incubation, the sample was washed three times with 1 mL PBS containing 1% FBS. Samples were incubated with a 1:500 dilution of streptavidin-Alexafluor 488 (1mg/mL) for 30 minutes on ice followed by a single wash with 1 mL PBS containing 1% FBS. Finally, the cells were examined under a fluorescence microscope. To conduct the blocking experiment, unlabeled cyclic peptide **29** and anti- $\alpha 3$ integrin antibody were incubated with the cells together with the biotinylated **29**. After the last wash, the cell pellet was resuspended in 100 μ L PBS and loaded into a cytospin centrifuge vial (Cytospin3, SHANDON). The cells were spun down onto slides at 2000 rpm for 2 min, mounted using DAPI-containing fluorescence mount solution (Invitrogen)

and examined under a fluorescence microscope (IX81, Olympus). (DAPI exposed with 1ms, Alexafluor 488 exposed with 100ms. Image Software: slidebook) Scale bar=5 μ m

Figure S3. *In Vivo* and *Ex Vivo* fluorescence imaging of orthotopic MDA-MB-231 xenograft



Tumor xenografts

Animal studies were performed according to a protocol approved by IACUC of the University of California, Davis. Female athymic nude mice (nu/nu), obtained from Harlan (Indianapolis, IN) at 5-6 weeks of age, were injected subcutaneously in the right 2-3 nipple fat pads with 5×10^6 MDA-MB-231 cells suspended in 200 μ L PBS. When the subcutaneous tumors reached 0.5 to 1.0cm in diameter or 40-50 days after implantation, the tumor-bearing mice were subjected to *in vivo* and *ex vivo* imaging studies.

In vivo and ex vivo optical imaging

Peptide ligand **29**-SA-Cy5.5(1.8 nmol), prepared by mixing 7.2 nmol of biotinylated **29** with 1.8 nmol of streptavidin-Cy5.5 in PBS overnight at 4°C, was injected via the tail vein in an anesthetized mouse before imaging. To conduct blocking experiments, an anti- α 3 integrin antibody was injected via tail vein 1 hour prior to injection of the optical probe. Animals were placed on a transparent sheet in the supine position. Images were acquired with a Kodak IS2000MM Image station (Rochester, NY) with a 625/20 band pass excitation filter, 700WA/35 band pass emission filter, and 150 W quartz halogen lamp light source set to maximum. Images were captured with a CCD camera set at F stop=0, FOV=150, and FP=0. For *ex vivo* imaging, the mice were euthanized and their organs were excised for imaging.

ESI-MS and purity for cyclic peptide 1 derivatives with substitution of Asp, Hyp, and Tyr(3-NO₂) (6-35)

6. MS(ESI) m/z: calcd for C₃₂H₄₂N₁₀O₁₆S₂ [M+1]⁺ 887.2, found 887.6. Purity 99%.
7. MS(ESI) m/z: calcd for C₃₂H₄₅N₁₁O₁₄S₂ [M+1]⁺ 872.2, found 872.7. Purity 98%.
8. MS(ESI) m/z: calcd for C₃₂H₄₃N₁₁O₁₄S₂ [M+1]⁺ 870.2, found 870.6. Purity 98%.
9. MS(ESI) m/z: calcd for C₃₂H₄₁N₁₁O₁₄S₂ [M+1]⁺ 868.2, found 868.5. Purity 97%.
10. MS(ESI) m/z: calcd for C₃₂H₄₂FN₁₁O₁₄S₂ [M+1]⁺ 888.2, found 888.6. Purity 98%.
11. MS(ESI) m/z: calcd for C₃₁H₄₁N₁₁O₁₄S₃ [M+1]⁺ 888.2, found 888.5. Purity 99%.
12. MS(ESI) m/z: calcd for C₃₈H₄₇N₁₁O₁₄S₂ [M+1]⁺ 946.2, found 946.6. Purity 98%.
13. MS(ESI) m/z: calcd for C₃₁H₄₁N₁₁O₁₄S₂ [M+1]⁺ 856.2, found 856.5. Purity 96%.
14. MS(ESI) m/z: calcd for C₃₃H₄₅N₁₁O₁₄S₂ [M+1]⁺ 884.2, found 884.6. Purity 98%.

15. MS(ESI) m/z: calcd for $C_{33}H_{46}N_{10}O_{13}S_2$ $[M+1]^+$ 855.2, found 855.6. Purity 97%.
16. MS(ESI) m/z: calcd for $C_{32}H_{43}ClN_{10}O_{13}S_2$ $[M+1]^+$ 875.2, found 875.5. Purity 98%.
17. MS(ESI) m/z: calcd for $C_{32}H_{43}IN_{10}O_{13}S_2$ $[M+1]^+$ 967.1, found 967.5. Purity 99%.
18. MS(ESI) m/z: calcd for $C_{32}H_{45}N_{11}O_{13}S_2$ $[M+1]^+$ 856.2, found 856.6. Purity 98%.
19. MS(ESI) m/z: calcd for $C_{33}H_{46}N_{10}O_{14}S_2$ $[M+1]^+$ 871.2, found 871.6. Purity 99%.
20. MS(ESI) m/z: calcd for $C_{32}H_{42}Br_2N_{10}O_{13}S_2$ $[M+1]^+$ 997.0, found 997.4. Purity 98%.
21. MS(ESI) m/z: calcd for $C_{32}H_{42}I_2N_{10}O_{13}S_2$ $[M+1]^+$ 1093.0, found 1093.3. Purity 97%.
22. MS(ESI) m/z: calcd for $C_{32}H_{42}N_{12}O_{17}S_2$ $[M+1]^+$ 931.2, found 931.5. Purity 98%.
23. MS(ESI) m/z: calcd for $C_{32}H_{43}FN_{10}O_{12}S_2$ $[M+1]^+$ 843.2, found 843.6. Purity 99%.
24. MS(ESI) m/z: calcd for $C_{32}H_{43}FN_{10}O_{12}S_2$ $[M+1]^+$ 843.2, found 843.5. Purity 99%.
25. MS(ESI) m/z: calcd for $C_{32}H_{43}FN_{10}O_{12}S_2$ $[M+1]^+$ 843.2, found 843.6. Purity 98%.
26. MS(ESI) m/z: calcd for $C_{33}H_{43}F_3N_{10}O_{12}S_2$ $[M+1]^+$ 893.2, found 893.6. Purity 98%.
27. MS(ESI) m/z: calcd for $C_{33}H_{43}F_3N_{10}O_{12}S_2$ $[M+1]^+$ 893.2, found 893.6. Purity 99%.
28. MS(ESI) m/z: calcd for $C_{32}H_{42}F_2N_{10}O_{12}S_2$ $[M+1]^+$ 861.2 found 861.5. Purity 98%.
29. MS(ESI) m/z: calcd for $C_{32}H_{42}F_2N_{10}O_{12}S_2$ $[M+1]^+$ 861.2, found 861.6 . Purity 97%.
30. MS(ESI) m/z: calcd for $C_{32}H_{41}F_3N_{10}O_{12}S_2$ $[M+1]^+$ 879.2, found 879.5. Purity 99%.
31. MS(ESI) m/z: calcd for $C_{32}H_{39}F_5N_{10}O_{12}S_2$ $[M+1]^+$ 915.2, found 915.5. Purity 98%.

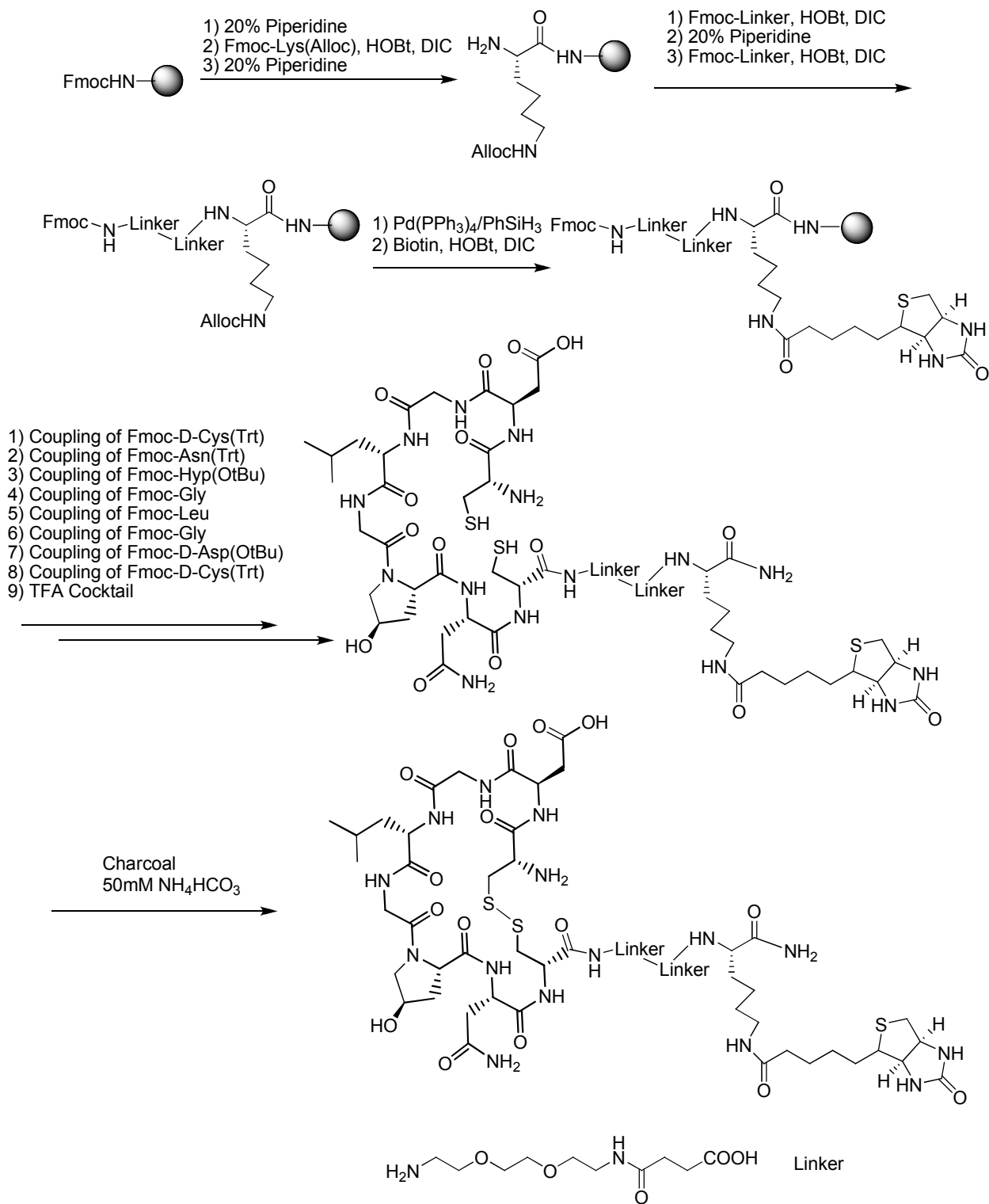
32. MS(ESI) m/z: calcd for $C_{32}H_{43}N_{11}O_{12}S_2$ $[M+1]^+$ 870.2 found 870.5. Purity 99%.
33. MS(ESI) m/z: calcd for $C_{32}H_{43}N_{11}O_{12}S_2$ $[M+1]^+$ 870.2, found 870.6. Purity 99%.
34. MS(ESI) m/z: calcd for $C_{32}H_{43}ClN_{10}O_{12}S_2$ $[M+1]^+$ 859.2, found 859.6. Purity 98%.
35. MS(ESI) m/z: calcd for $C_{33}H_{43}N_{11}O_{12}S_2$ $[M+1]^+$ 850.2, found 850.6. Purity 98%.

ESI-MS and purity for cyclic peptide 1 derivatives with N-terminal modification (36-58)

36. MS(ESI) m/z: calcd for $C_{36}H_{51}N_{11}O_{15}S_2$ $[M+1]^+$ 942.3, found 942.7. Purity 99%.
37. MS(ESI) m/z: calcd for $C_{38}H_{55}N_{11}O_{15}S_2$ $[M+1]^+$ 970.3, found 970.9. Purity 99%.
38. MS(ESI) m/z: calcd for $C_{44}H_{67}N_{11}O_{15}S_2$ $[M+1]^+$ 1054.4, found 1055.0. Purity 98%.
39. MS(ESI) m/z: calcd for $C_{41}H_{53}N_{11}O_{15}S_2$ $[M+1]^+$ 1004.3, found 1004.8. Purity 97%.
40. MS(ESI) m/z: calcd for $C_{50}H_{63}N_{11}O_{15}S_2$ $[M+1]^+$ 1122.40, found 1122.9. Purity 99%.
41. MS(ESI) m/z: calcd for $C_{39}H_{55}N_{11}O_{15}S_2$ $[M+1]^+$ 982.3, found 982.9. Purity 96%.
42. MS(ESI) m/z: calcd for $C_{38}H_{48}N_{12}O_{15}S_2$ $[M+1]^+$ 977.2, found 977.8. Purity 98%.
43. MS(ESI) m/z: calcd for $C_{39}H_{48}N_{12}O_{15}S_2$ $[M+1]^+$ 1021.3, found 1021.8. Purity 99%.
44. MS(ESI) m/z: calcd for $C_{39}H_{47}F_2N_{11}O_{15}S_2$ $[M+1]^+$ 1012.3, found 1012.7. Purity 98%.
45. MS(ESI) m/z: calcd for $C_{41}H_{54}N_{12}O_{15}S_2$ $[M+1]^+$ 1019.3, found 1019.6. Purity 97%.
46. MS(ESI) m/z: calcd for $C_{40}H_{48}F_3N_{11}O_{15}S_2$ $[M+1]^+$ 1044.3, found 1044.7. Purity 98%.
47. MS(ESI) m/z: calcd for $C_{36}H_{46}N_{12}O_{15}S_3$ $[M+1]^+$ 983.2, found 983.7. Purity 99%.
48. MS(ESI) m/z: calcd for $C_{45}H_{53}N_{11}O_{15}S_2$ $[M+1]^+$ 1052.3 found 1052.8. Purity 99%.

- 49.** MS(ESI) m/z: calcd for $\text{C}_{37}\text{H}_{52}\text{N}_{12}\text{O}_{16}\text{S}_2$ $[\text{M}+1]^+$ 985.3, found 985.7. Purity 98%.
- 50.** MS(ESI) m/z: calcd for $\text{C}_{36}\text{H}_{50}\text{N}_{12}\text{O}_{16}\text{S}_2$ $[\text{M}+1]^+$ 971.3, found 971.7. Purity 96%.
- 51.** MS(ESI) m/z: calcd for $\text{C}_{39}\text{H}_{54}\text{N}_{12}\text{O}_{16}\text{S}_2$ $[\text{M}+1]^+$ 1011.2, found 1011.7. Purity 99%.
- 52.** MS(ESI) m/z: calcd for $\text{C}_{39}\text{H}_{48}\text{N}_{12}\text{O}_{16}\text{S}_2$ $[\text{M}+1]^+$ 1005.3, found 1005.7. Purity 98%.
- 53.** MS(ESI) m/z: calcd for $\text{C}_{39}\text{H}_{47}\text{ClN}_{12}\text{O}_{16}\text{S}_2$ $[\text{M}+1]^+$ 1039.2, found 1039.7. Purity 98%.
- 54.** MS(ESI) m/z: calcd for $\text{C}_{45}\text{H}_{52}\text{N}_{12}\text{O}_{16}\text{S}_2$ $[\text{M}+1]^+$ 1081.3, found 1081.5. Purity 97%.
- 55.** MS(ESI) m/z: calcd for $\text{C}_{41}\text{H}_{52}\text{N}_{12}\text{O}_{18}\text{S}_2$ $[\text{M}+1]^+$ 1065.3, found 1065.7. Purity 97%.
- 56.** MS(ESI) m/z: calcd for $\text{C}_{40}\text{H}_{47}\text{F}_3\text{N}_{12}\text{O}_{16}\text{S}_2$ $[\text{M}+1]^+$ 1074.3, found 1073.7. Purity 96%.
- 57.** MS(ESI) m/z: calcd for $\text{C}_{39}\text{H}_{47}\text{N}_{13}\text{O}_{18}\text{S}_2$ $[\text{M}+1]^+$ 1050.3, found 1050.7. Purity 98%.
- 58.** MS(ESI) m/z: calcd for $\text{C}_{41}\text{H}_{50}\text{N}_{12}\text{O}_{18}\text{S}_2$ $[\text{M}+1]^+$ 1063.3, found 1063.7. Purity 98%.

Scheme S3. Solid phase synthesis of biotinylated LXY1



Rink amide resin (1g, loading 0.6mmol/g) was swollen in DMF for 2h before Fmoc deprotection with 20% piperidine in DMF twice (5min, 15min). The beads were washed sequentially with DMF, MeOH, DCM, MeOH, and DMF, three times each. A solution of Fmoc-Lys(Alloc)-OH (3 equiv), HOBt (3 equiv.) and DIC (3 equiv.) in DMF were added to the beads. The coupling was carried out at room temperature until a Kaiser test was negative. The beads were washed with DMF three times, followed by Fmoc deprotection. The beads were then subjected to two cycles of coupling of an Fmoc-linker. The Alloc protection group was removed with Pd(Ph₃)₄ (0.24 equiv) and PhSiH₃ (20 equiv) in DCM with agitation for 30min, twice. After washing, a solution of biotin (3equiv), HOBt (3equiv), and DIC (3equiv) in DMF was added to the beads. The reaction proceeded at room temperature until a Kaiser test was negative. After Fmoc deprotection, the beads were subjected to stepwise assembly of Fmoc-D-Cys(Trt)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Hyp(OtBu)-OH, Fmoc-Gly-OH, Fmoc-Leu-OH, Fmoc-Gly-OH, Fmoc-D-Asp(OtBu)-OH, and Fmoc-D-Cys(Trt)-OH using Fmoc solid-phase chemistry. Finally, the beads were sequentially washed with DMF, MeOH, DCM, and then dried under vacuum. The dried beads were treated with a TFA cocktail containing 82.5% TFA: 5% phenol: 5% thioanisole: 5% H₂O: 2.5% triisopropylsilane (TIS), at room temperature for 3h. The cleavage solution was collected, concentrated, and precipitated in cold diethyl ether. The crude peptides were dissolved in 100mL 50mM NH₄HCO₃ buffer, followed by the addition of 100mg activated charcoal. The solution was stirred at room temperature until the Ellman test was negative. The reaction solution was filtered, collected and lyophilized. The crude product was purified by preparative reverse-phase high performance liquid chromatography (RP-HPLC). The resultant solids were characterized by ESIMS. ESI-MS m/z: calcd for C₆₅H₁₀₈N₁₈O₂₃S₃ [M+1]⁺ 1605.7, found 1066.0. Purity 99%.

The Biotinylated **29** (cdGPhe(3,5-2F)GHypNc-Linker-Linker-Lys(Biotin) was synthesized in the same manner as described above. ESI-MS m/z: calcd for C₆₈H₁₀₄N₁₈O₂₇S₃ [M+1]⁺, 1675.6, found 1675.9. Purity 98%.