General. N-succinimidyl-4-fluorobenzoate (SFB) was obtained from ABX GmbH (Radeberg, Germany). All 9-fluorenylmethyloxycarbonyl (Fmoc) protected amino acids were purchased from Novabiochem/EMD Chemicals Inc (La Jolla, CA) or CS Bio (Menlo Park, CA). Phosphate buffered saline (PBS) was from Gibco/Invitrogen (Carlsbad, CA). EGF, N-(2-minoethyl)maleimide trifluoroacetate salt, N, N'diisopropylethylamine (DIPEA) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). All other chemicals were purchased from Thermo Fisher Scientific (Fair Lawn, NJ) unless otherwise specified. The A431, HT29 and U87MG human cancer cell lines were obtained from American Type Culture Collection (Manassas, VA). The human HNSCC cell lines SQB20, SAS, and UM-SCC-22B were obtained from Dr. J. Martin Brown's laboratory at Stanford University, the Cell Resource Center for the Biomedical Research Institute of Development, Aging, and Cancer, Tohoku University, and from the University of Michigan, respectively. Nude mice (nu/nu, female 5-6 weeks old) were purchased from Charles River Laboratory (Wilmington, MA). A CRC-15R PET dose calibrator (Capintec Inc., Ramsey, NJ) was used for all radioactivity measurements. Reverse phase high performance liquid chromatography (RP-HPLC) was performed on a Dionex Summit HPLC system (Dionex Corporation, Sunnyvale, CA) equipped with a 170U 4-Channel UV-Vis absorbance detector and radioactivity detector (Carroll & Ramsey Associates, model 105S, Berkeley, CA). UV detection wavelengths were 218 nm, 254 nm and 280 nm for all the experiments. Both semipreparative (Vydac, Hesperia, CA. 218TP510-C18, 10 mm × 250 mm) and analytical (Dionex, Sunnyvale, CA. Acclaim120 C18, 4.6 mm × 250 mm) RP-HPLC columns were used. The mobile phase was solvent A, 0.1% trifluoroacetic acid (TFA)/H₂O, and solvent B, 0.1%TFA/acetonitrile. Matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS) were performed on a Perseptive Voyager-DE RP Biospectrometry instrument (Framingham, MA) by the Stanford Protein and Nucleic Acid Biotechnology Facility (Stanford, CA).

For dynamic scanning, approximately 4.81 MBq (130 μ Ci) of ¹⁸F-FBEM-Cys-Z_{EGFR:1907} was co-injected with 45 μ g of Ac-Cys-Z_{EGFR:1907} into Nu/Nu mice via the tail vein and a 35 min duration dynamic scan (5 × 20 sec, 5 × 40 sec, 10 × 1 min, 8 × 2.5 min; total of 28 frames) was started right after injection of the probe. The time activity curves (TACs) were drawn on major tissue/organs such as muscle, kidney, liver and blood (Supplemental Fig. 1).



Supplemental Figure 1

All the static microPET images were reconstructed by a two-dimensional orderedsubsets expectation maximization (OSEM) algorithm. No background correction was performed. Region of interests (ROIs; 5 pixels for coronal and transaxial slices) were drawn over the tumor on decay-corrected whole-body coronal images. Tumors uptakes (max %ID/g) were also calculated from ROIs (Supplemental Fig. 2A, 2B). The tumor/liver uptake ratio was calculated from the mean value (Supplemental Fig. 2C).



Supplemental Figure 2

Purified peptide Ac-Cys- $Z_{EGFR:1907}$ was dissolved in water, then concentration and composition were determined by Hitachi amino acid analyzer (Molecular Structure Facility, Davis, CA) (Supplemental Fig.3).

Sample:	Zen Cheng		Init. Vol(uL):	50	
Int.Std.	2.124		Run #:	267	
	2.000		Final Vol(ul):	150	
Amino Acid	nmoles	<u>nms/50 ul</u>	<u>mole %</u>	<u>N (data)</u>	<u>N (exp)</u>
Asx	10.533	9.918	19.01	11.2	11
Thr	0.958	0.902	1.73	1.0	1
Ser	2.782	2.620	5.02	3.0	3
Glx	6.915	6.511	12.48	7.4	7
Pro	2.940	2.768	5.31	3.1	3
Gly	1.051	0.990	1.90	1.1	1
Ala	9.069	8.540	16.37	9.7	9
Val	1.887	1.777	3.41	2.0	2
lle	1.896	1.785	3.42	2.0	2
Leu	5.993	5.643	10.82	6.4	6
Tyr	0.000	0.000	0.00	0.0	0
Phe	1.859	1.750	3.36	2.0	2
His	0.000	0.000	0.00	0.0	0
Lys	4.856	4.573	8.76	5.2	5
Arg	0.965	0.909	1.74	1.0	1
Cysteic	0.849	0.799	1.53	0.9	1
Methionine	1.890	1.780	3.41	2.0	2
Trp	0.960	0.904	1.73	1.0	3
Totals:		52.169	100.00		59
nmol peptio	de/50 ul:	0.884	<u>Total nmol:</u>	2.6526381	
Concentration:			0.053	nmol/uL	
M.W. calc.	6647.54	dalton			
Total Protein	17.634	ug			
	0.353	ug/ul			JS 01/17/2012

Supplemental Figure 3