# Supplemental Informations for Identification and Characterization of a Suite of Tumor Targeting Peptides for Non-Small Cell Lung Cancer

Michael J. McGuire<sup>1,4</sup>, Bethany Powell Gray<sup>1,4</sup>, Shunzi Li<sup>1,4</sup>, Dorothy Cupka<sup>1,4</sup>, Lauren Averett Byers<sup>6</sup>, Lei Wu<sup>1,4</sup>, Shaghayegh Rezaie<sup>1,4</sup>, Ying-Horng Liu<sup>1,4</sup>, Naveen Pattisapu<sup>1,4</sup>, James Issac<sup>1,4</sup>, Tsukasa Oyama<sup>1</sup>, Lixia Diao<sup>6</sup>, John V. Heymach<sup>6</sup>, Xian-Jin Xie<sup>3,4</sup>, John D. Minna <sup>1,2,4,5</sup>, and Kathlynn C. Brown<sup>1,4</sup>



**Supplemental Figure S1. The H1299.2 peptide can be truncated to a 15-mer with no loss of binding activity.** A. A series of peptides were synthesized in which 5-amino acid blocks were replaced with alanine residues. The ability of the peptides to block uptake of H1299.2 phage as described in the text. Changing the c-terminal 5 amino acids to alanine does not affect the ability of the peptide to compete with phage binding while the other 3 peptides are nonfunctional. The half maximal binding affinity of the 15-mer is the same as the full length parental peptide.

# No primary antibody control



**Mouse Anti-phage antibody** 



No phage

**Control phage** 

H2009.1 phage

H460.1 phage

**Supplementary Figure S2. The H460.1 phage displays similar binding patterns as the free H460.1 tetrameric peptide.** MCF-7 cells were seeded on 8 well chamber slide and cultured overnight. Cell were incubated for 10 minutes with the indicated phage clone at 1 x 10<sup>8</sup> cfu/ml or without phage. Unbound phage were removed and cells were washed 4 times with PBS<sup>+</sup>/0.1%BSA followed by 2 HCI-Glycine rinses. Cells were washed with PBS and fixed in PBS-buffered formalin. Samples were permeabilized and treated with 2% goat serum and 2% BSA in PBS containing 0.1% Triton X-100 (Perm and Block Buffer). After 10 minutes in Perm and Block Buffer, samples were incubated for 30 min as indicated with 1:100 dilution of mouse anti-phage antibody (in-house generated antibody). Samples were washed 3 times with PBS <sup>+</sup>/0.1% BSA (5 min each) prior to incubation (30 min) with 1:200 dilution of goat ant-mouse IgG-Alexafluor 488 conjugate (Invitrogen) in Perm and Block Buffer. Samples were washed 3 times with PBS<sup>+</sup>/0.1% BSA followed by 2 PBS only washes. Cells were counterstained with 10µg/ml wheat germ agglutinin-Texas Red conjugate for 10 min followed by 2 PBS rinses. Samples were overlaid with Vectashield + DAPI prior to fluorescence microscopy. Scale bars represent 20 microns.



Supplementary Figure S3. There is a linear correlation between of binding scores of the triplet peptide group, H1299.2, HCC15.1, and HCC15.2. Binding scores were generated as described in the manuscript. R<sup>2</sup> values are indicated.



Supplemental Figure S4. Peptide Binding of the triplet cluster correlates to expression levels of three proteins. Cells were dichotomized into high and low binders of H1299.2, HCC15.1, and HCC15.2 using a cell binding score as 5000 as a benchmark and compared to expression levels of 200 proteins analyzed by RPPA (t test analysis)



Supplemental Figure S5. The isolated NSCLC-binding peptides do not affect cell growth. Cells were plated in 96 well plates at concentrations of 1,000 cells/well (460 and 1299 cells) or 2,000 cells per well (2009 cells). Peptides were added to the cell media 24 h later at the indicated concentration. Cell viability was determined using the CellTiter-Glo<sup>®</sup> Luminescent Assay per manufacturer's protocol at 72 hrs.

#### Table S-I Selectivity of Phage Clones on NSCLC cell lines

	H1299.1	H1299.2	H2009.1	H460.1	A549.1	HCC15.1	HCC15.2	HCC95.1	H1155.1	H1155.2	H661.1
A549 (lung AD)	12 ± 2.8	17 ± 3.9	9.2 ± 1.6	14 ± 1.2	21 ± 2.7	1.1 ± 0.32	33 ± 5.5	1.1 ± 0.32	5 ± 2.7	5.4 ± 3.8	1.9 ± 0.46
Calu-1 (lung mucoepidermoid)	45 ± 17	92 ± 19	0.53 ± 0.14	3.8 ± 1.4	2.8 ± 0.48	1.4 ± 0.39	28 ± 9.4	1.3 ± 0.31	4.2 ± 1.6	1.5 ± 0.66	2.0 ± 0.46
Calu-3 (lung AD)	1.2 ± 0.58	1.6 ± 0.29	34 ± 13	1.0 ± 0.59	1.6 ± 1.1	0.73 ± 0.11	1.4 ± 0.98	2.8 ± 0.8	12 ± 6	3.0 ± 0.62	2.8 ± 2.3
H1155 (lung LC)	3.3 ± 1.7	1.2 ± 0.44	2.6 ± 0.49	6.6 ± 2.2	2.2 ± 1.4	0.56 ± 0.19	1.7 ± 0.61	2.1 ± 1.2	55 ± 21	61 ± 7.9	2.7 ± 2.1
HCC1171 (lung carcinoma)	27 ± 3.1	35 ± 6.9	2.9 ± 1.5	0.38 ± 0.23	0.47 ± 0.27	4.3 ± 1.7	45 ± 2.9	1.0 ± 0.27	1.9 ± 1.0	3.3 ± 1.6	1.0 ± 0.12
H1264 (lung AD)	2.6 ± 0.69	7.3 ± 2.6	1.3 ± 0.8	2.7 ± 2.1	0.36 ± 0.21	3.9 ± 1.9	66 ± 13	0.83 ± 0.24	2.8 ± 1.7	3.2 ± 1.3	1.2 ± 0.36
H1299 (lung LC)	190 ± 20	83 ± 17	4.7 ± 0.23	6.3 ± 2.8	11.3 ± 3.3	170 ± 75	100 ± 37	1.6 ± 0.59	3.1 ± 1.1	5.1 ± 2.6	3.7 ± 1.8
H1355 (lung AD)	6.4 ± 3.9	4.5 ± 3.2	2.9 ± 0.92	2.5 ± 0.66	2.8 ± 1.9	4.3 ± 2.6	25 ± 3.8	2.8 ± 0.8	5.8 ± 2.6	3.8 ± 1.8	0.99 ± 0.21
H1437 (lung AD)	1.8 ± 1.2	3.6 ± 1.3	2.3 ± 0.96	3.3 ± 1.2	3.4 ± 1.2	3.6 ± 1.5	0.95 ± 0.24	2.5 ± 1.2	12 ± 2.6	8.5 ± 3.3	2.1 ± 0.55
H157 (lung SQ)	14 ± 4.6	3.7 ± 0.7	8.7 ± 4.1	2.3 ± 1.3	3.2 ± 2.7	10 ± 5.3	14 ± 9.5	0.71 ± 0.2	1.1 ± 0.62	2.4 ± 0.68	2.6 ± 2.2
H1648 (lung AD, lymph node met)	1.4 ± 0.22	14 ± 2	230 ± 99	6.6 ± 1.2	4.1 ± 2	1.1 ± 0.26	17 ± 5.3	3.3 ± 1.1	3.5 ± 0.47	1.3 ± 0.38	1.2 ± 0.48
H1650 (Lung AD/BAC)	0.55 ± 0.14	2.0 ± 0.3	15 ± 5.5	1.5 ± 0.64	1.4 ± 0.35	0.34 ± 0.071	0.22 ± 0.13	0.87 ± 0.71	4.4 ± 2.8	3 ± 1.3	0.65 ± 0.27
H1666 (lung AD/BAC)	0.84 ± 0.25	0.86 ± 0.15	34 ± 10	7 ± 3.8	9.4 ± 1.2	0.42 ± 0.092	5 ± 2.1	0.41 ± 0.072	2.1 ± 1	1.7 ± 0.18	0.97 ± 0.42
H1819 (lung AD, lymph node met)	0.97 ± 0.53	8.6 ± 4.1	250 ± 30	6.4 ± 1.9	3.1 ± 2.7	0.96 ± 0.098	10 ± 1.7	2.2 ± 1.8	2.9 ± 1.3	1.5 ± 0.75	4.4 ± 2.7
H1975 (lung AD)	0.39 ± 0.16	0.57 ± 0.25	120 ± 17	0.93 ± 0.22	3.4 ± 1.3	0.46 ± 0.14	3.5 ± 0.98	0.55 ± 0.072	3.6 ± 1.4	1.2 ± 0.65	1.0 ± 0.12
H1993 (AD, lymph node met of 1993)	27 ± 6	100 ± 16	37 ± 7.9	3.3 ± 2.2	4 ± 2.3	32 ± 10	1.2 ± 0.2	39 ± 15	2.8 ± 1.4	2.7 ± 1.4	1.2 ± 0.2.0
H2009 (lung AD, lymph node met)	31 ± 14	310 ± 140	300 ± 1.8	6.2 ± 2.4	2.2 ± 0.96	68 ± 21	36 ± 24	0.56 ± 0.26	4 ± 1.6	9.6 ± 6.8	4.3 ± 2.9
H2073 (lung AD)	21 ± 5.5	25 ± 3.6	54 ± 18	2.8 ± 0.99	0.74 ± 0.33	60 ± 20	9.3 ± 2.8	0.87 ± 0.43	1.5 ± 0.81	2.8 ± 1.4	1.2 ± 0.82
H2086 (lung AD)	3.6 ± 1.4	7.8 ± 4	380 ± 120	6.9 ± 2.4	4.1 ± 1	1.3 ± 1.1	14 ± 3.3	0.57 ± 0.32	2.7 ± 0.55	2.7 ± 0.73	0.85 ± 0.18
H2087 (lung AD, lymph node met)	39 ± 13	79 ± 42	2.9 ± 1.4	6.0 ± 3.6	4.2 ± 0.94	1.3 ± 1.1	14 ± 3.3	0.57 ± 0.32	2.7 ± 0.55	2.7 ± 0.73	0.85 ± 0.18
H2122 (lung AD)	1.7 ± 0.39	3.6 ± 1.3	48 ± 6	5.1 ± 1.7	4.8 ± 1.5	0.56 ± 0.14	5.9 ± 1.6	0.53 ± 0.099	3.5 ± 1.9	2.5 ± 1.7	$1.2 \pm 0.34$
H2126 (lung AD)	0.91 ± 0.55	11 ± 5.8	28 ± 10	0.23 ± 0.1	7.1	$3.9 \pm 0.4$	3.2 ± 1.5	0.37 ± 0.035	1.3 ± 0.7	4.7 ± 0.35	0.28 ± 0.15
H2347 (lung AD)	0.79 ± 0.46	0.33 ± 0.12	38 ± 9.2	0.99 ± 0.57	0.67 ± 0.31	2 ± 0.87	7.8 ± 1.8	0.76 ± 0.26	5.5 ± 3.4	3.7 ± 1.8	1.1 ± 0.38
H2882 (lung carcinoma)	1.5 ± 0.36	1.1 ± 0.56	1.4 ± 0.22	0.54 ± 0.17	0.4 ± 0.11	0.91 ± 0.39	1.6 ± 0.89	3.3 ± 2.7	6.0 ± 1.4	5.1 ± 1.0	1.6 ± 0.89
H322( Lung AD/BAC)	6.2 ± 2.5	10 ± 3.8	68 ± 17	1.1 ± 0.33	0.71 ± 0.23	3.8 ± 1.1	17 ± 1.5	2.5 ± 1.5	20 ± 1.7	12 ± 2.1	3.6 ± 1.9
H358 (Lung AD/BAC)	29 ± 6.6	43 ± 13	130 ± 19	2.2 ± 1.1	0.9 ± 0.29	6.7 ± 3.2	13 ± 3.9	3.8 ± 3	1.9 ± 0.89	2.6 ± 1.7	1.4 ± 0.6
H441(lung AD/BAC)	2.4 ± 0.94	1.8 ± 0.73	19 ± 4.4	0.95 ± 0.38	0.8 ± 0.38	1.7 ± 1.4	2.3 ± 0.82	1.1 ± 0.093	4.6 ± 1.4	3.3 ± 1.2	3.8 ± 1.7
H460 (lung LC)	3.2 ± 0.81	1.1 ± 0.33	2 ± 0.45	120 ± 25	150 ± 35	0.83 ± 0.21	0.64 ± 0.49	6.9 ± 4.8	3.2 ± 1.1	1.4 ± 0.91	0.54 ± 0.08
H661 (lung LC)	2.8 ± 1	2.8 ± 2	5.9 ± 1.9	2.4 ± 0.8	2.2 ± 0.5	22 ± 1.5	10 ± 1.7	0.88 ± 0.3	2.5 ± 1.1	6.1 ± 2.4	26 ± 5.8
HCC827 (lung AD)	1.9 ± 0.66	2.2 ± 1.4	180 ± 66	4.1 ± 0.99	6.3 ± 2.5	3.2 ± 1.6	2.2 ± 0.12	0.75 ± 0.39	5.4 ± 1.9	14 ± 2.3	6.8 ± 2.5
HCC1359 (lung SQ)	2.1 ± 1.2	3.1 ± 0.75	5.4 ± 1.3	1.2 ± 0.44	0.72 ± 0.36	0.8 ± 0.2	1.2 ± 0.53	0.8 ± 0.35	2.9 ± 1.1	3.9 ± 1.6	1.0 ± 0.3
HCC15 (lung SQ)	16 ± 2.6	16 ± 1.4	1.0 ± 0.51	7.6 ± 3	14 ± 4.2	44 ± 18	220 ± 32	0.67 ± 0.16	1.3 ± 0.52	5.6 ± 1.5	5.9 ± 1.2
HCC2279 (lung AD)	13 ± 6.9	3.9 ± 1.8	3.1 ± 2	0.64 ± 0.39	1.4 ± 0.6	2.8 ± 0.56	20 ± 3.8	2.5 ± 1.3	1.2 ± 0.18	2.8 ± 1.0	1.4 ± 0.54
HCC2450 (lung SQ)	2.6 ± 0.97	3.5 ± 0.48	3.1 ± 0.92	1.7 ± 0.8	1.5 ± 0.65	0.86 ± 0.52	2.4 ± 1.7	0.46 ± 0.15	1.5 ± 0.21	3.4 ± 1.1	0.68 ± 0.17
HCC366 (lung adenosquamous)	8.5 ± 4	19 ± 12	3.5 ± 1.4	1.8 ± 0.59	1.2 ± 0.49	2.3 ± 1.8	21 ± 2.3	0.72 ± 0.19	1.4 ± 0.72	1.9 ± 1.1	0.61 ± 0.26
HCC461 (lung AD)	1.8 ± 0.64	3.4 ± 2	10 ± 5.8	1.6 ± 0.75	1.9 ± 0.54	4.2 ± 1.7	25 ± 8.6	0.48 ± 0.07	4.2 ± 1.7	5.7 ± 1.6	$0.5 \pm 0.52$
HCC515 (lung AD)	5.3 ± 3.4	1.7 ± 0.29	95 ± 29	0.61 ± 0.18	0.43 ± 0.1	2.2 ± 0.68	13 ± 3.9	1.7 ± 0.42	2.2 ± 0.68	0.86 ± 0.25	$1.3 \pm 0.4$
HCC78 (lung AD)	0.96 ± 0.56	3.8 ± 0.4	95 ± 21	0.51 ± 0.2	0.84 ± 0.39	1.6 ± 0.8	1.8 ± 0.75	0.97 ± 0.18	3.6 ± 0.93	1.5 ± 0.69	1.6 ± 0.35
HCC95 (lung SQ)	$1.6 \pm 0.55$	$1.2 \pm 0.66$	$1.8 \pm 0.74$	1.8 ± 0.3	2.9 ± 1.1	$0.66 \pm 0.22$	$1.2 \pm 0.34$	20 + 2.7	1.1 ± 0.27	2.5 ± 1.2	$1.9 \pm 1.0$
PC9 (lung AD)	1.9 ± 0.62	5.8 ± 2.2	47 ± 16	0.22 ± 0.05	0.56 ± 0.29	2.5 ± 1.2	32 + 8.5	$0.46 \pm 0.19$	3.2 ± 0.88	$6.3 \pm 4.0$	1.3 ± 0.52
BEAS-2B (Normal Control)	4.2 ± 0.40	3.1 ± 0.67	4.1 ± 2.0	0.51 ± 0.23	2.7 ± 1.3	1.3 ± 1.2	2.7 ± 0.81	0.29 ± 0.18	1.4 ± 0.79	2.4 ± 1.2	$0.29 \pm 0.14$
Total Number of Cell Lines with Selectivity $\ge$ 20		8	8	18	1	2	6	13	2	2	1 1

Selectivity ≥ 20 are indicated in red for clarity Errors are represpented as S.E.M.

# Table S-II Selectivity of Phage Clones on Other Cancer Cell Lines

	H1299.1	H1299.2	H2009.1	H460.1	A549.1	HCC15.1	HCC15.2	HCC95.1	H1155.1	H1155.2	H661.1	
MCF7(breast)	1.5 ± 1.1	2.6 ± 2.3	15 ± 3.8	530 ± 170	50 ± 15	4.2 ± 3	1.7 ± 0.33	1.1 ± 0.14	2.6 ± 0.55	5.6 ± 2.1	2.9 ± 0.97	-
T47D (breast)	10 ± 2.2	12 ± 2.3	0.57 ± 0.038	0.82 ± 0.024	1.2 ± 0.19	24 ± 6.0	14 ± 3.1	2.7 ± 1.8	5.1 ± 0.71	4.7 ± 1.3	4.3	
MDA-MB-468 (breast)	1.5 ± 0.24	2.6 ± 0.97	25 ± 7.6	1.9 ± 0.22	3.8 ± 1.1	0.59 ± 0.42	0.83 ± 0.54	ND	0.25 ± 0.13	0.78 ± 0.60	ND	
MDA-MB-231 (breast)	28 ± 13.3	16 ± 6.1	1 ± 0.23	2.4 ± 1.1	0.94 ± 0.26	170 ± 78	150 ± 38	1.4 ± 0.91	3.3 ± 1.1	8.5 ± 1.9	3.3 ± 1.6	
PC3 (prostate)	4.2 ± 1.6	12 ± 2.4	0.8 ± 0.26	1.7 ± 0.56	0.51 ± 0.13	6.6 ± 0.87	5.1 ± 0.8	6.7 ± 4.2	5.1 ± 4	11 ± 4.7	0.71 ± 0.17	
LnCap (carcinoma)	1.8 ± 1.1	0.31 ± 0.27	1.3 ± 0.71	0.44 ± 0.17	0.13 ± 0.06	1.9 ± 1.2	4.8 ± 1.3	2.3 ± 1.4	7.8 ± 3.7	23 ± 0.7	0.86 ± 0.51	
SCOV3 (ovarian)	2 ± 0.59	6.1 ± 3.4	7.7 ± 4.9	1.9 ± 0.52	0.97 ± 0.37	2.7 ± 1.1	51 ± 17	0.78 ± 0.44	1.1 ± 0.23	2.3 ± 1.1	0.21 ± 0.0088	
MiaPaCa (pancreatic)	20 ± 5.3	90 ± 20	1.6 ± 1	0.53 ± 0.29	0.89 ± 0.29	3.7 ± 1.9	35 ± 4.1	0.57 ± 0.16	0.44 ± 0.29	1.2 ± 0.41	1.7 ± 0.97	
Panc1 (pancreatic)	19 ± 6.6	44 ± 12	1.4 ± 0.5	0.85 ± 0.28	0.66 ± 0.33	9.0 ± 3.0	15 ± 3.5	0.77 ± 0.23	2.0 ± 0.40	2.2 ± 0.40	1.4 ± 0.51	
SW480 (colorectal)	4.5 ± 2.2	9.1 ± 2	6.6 ± 3.3	2.1 ± 0.55	3.5 ± 0.75	61 ± 2.7	120 ± 12	0.82 ± 0.39	2 ± 0.73	14 ± 1.3	0.77 ± 0.38	
HUH7 (liver)	200 ± 60	290 ± 65	2.9 ± 1.6	330 ± 95	250 ± 72	210 ± 53	140 ± 40	2.2 ± 0.53	3.3 ± 2.1	2.1 ± 0.86	2.2 ± 0.67	
Total Number of Cell Lines												
with Selectivity ≥ 20		3	3	1	2	2	3	5 (	) (		1	0
Selectivity ≥ 20 are indicated												

in red for clarity Errors are represpented as S.E.M.

#### Table S-III Binding Analysis of Peptides H1299.2 Monomer

Normalized Binding log(inhibitor) vs. normalized response -- Variable slope

Best-fit values	
LogIC50	-7.687
HillSlope	-1.251
IC50	2.057E-08
Std. Error	
LogIC50	0.1235
HillSlope	0.3508
95% Confidence Intervals	
LogIC50	-7.954 to -7.420
HillSlope	-2.009 to -0.4933
IC50	1.113e-008 to 3.801e-008
Goodness of Fit	
Degrees of Freedom	13
R square	0.8755
Absolute Sum of Squares	2506
Sy.x	13.88
Number of points	
Analyzed	15

#### H1299.2 Tetramer

log(inhibitor) vs. normalized response -- Variable slope Best-fit values LogIC50 -9.918 HillSlope -0.5365 IC50 1.209E-10 Std. Error LogIC50 0.2429 HillSlope 0.1706 95% Confidence Intervals LogIC50 -10.43 to -9.407 HillSlope -0.8950 to -0.1780 IC50 3.731e-011 to 3.915e-010 Goodness of Fit Degrees of Freedom 18 R square 0.6083 Absolute Sum of Squares 4960 16.6 Sy.x Number of points Analyzed 20

#### H2009.1 Monomer

log(inhibitor) vs. normalized response -- Variable slope

Best-fit values	
LogIC50	-7.666
HillSlope	-1.234
IC50	2.158E-08
Std. Error	
LogIC50	0.1829
HillSlope	0.4716
95% Confidence Intervals	
LogIC50	-8.044 to -7.288
HillSlope	-2.210 to -0.2585
IC50	9.029e-009 to 5.157e-008
Goodness of Fit	
Degrees of Freedom	23
R square	0.7462
Absolute Sum of Squares	14061
Sy.x	24.73
Number of points	
Analyzed	25







### H2009.1 Tetramer

log(inhibitor) vs. normalized response -- Variable slope

Best-fit values	
LogIC50	-9.463
HillSlope	-0.7789
IC50	3.441E-10
Std. Error	
LogIC50	0.09652
HillSlope	0.1319
95% Confidence Intervals	
LogIC50	-9.670 to -9.256
HillSlope	-1.062 to -0.4960
IC50	2.136e-010 to 5.543e-010
Goodness of Fit	
Degrees of Freedom	14
R square	0.9018
Absolute Sum of Squares	1248
Sy.x	9.443
Number of points	
Analyzed	16



### H1299.1 Monomer

log(inhibitor) vs. normalized response -- Variable slope Best-fit values

Dest-iii values	
LogIC50	-5.716
HillSlope	-0.6501
IC50	0.000001923
Std. Error	
LogIC50	0.1658
HillSlope	0.2073
95% Confidence Intervals	
LogIC50	-6.122 to -5.310
HillSlope	-1.157 to -0.1429
IC50	7.558e-007 to 4.894e-006
Goodness of Fit	
Degrees of Freedom	6
R square	0.7532
Absolute Sum of Squares	1290
Sy.x	14.66
Number of points	
Analyzed	8



log(inhibitor) vs. normalized response -- Variable slope

Best-fit values	
LogIC50	-7.377
HillSlope	-1.198
IC50	4.202E-08
Std. Error	
LogIC50	0.1019
HillSlope	0.2685
95% Confidence Intervals	
LogIC50	-7.604 to -7.149
HillSlope	-1.796 to -0.5995
IC50	2.491e-008 to 7.088e-008
Goodness of Fit	
Degrees of Freedom	10
R square	0.887
Absolute Sum of Squares	1456
Sy.x	12.07
Number of points	
Analyzed	12





## HCC15.1 Monomer

log(inhibitor) vs. normalized re	sponse
Best-fit values	
LogIC50	-6.409
IC50	3.897E-07
Std. Error	
LogIC50	0.1337
95% Confidence Intervals	
LogIC50	-6.693 to -6.126
IC50	2.028e-007 to 7.485e-007
Goodness of Fit	
Degrees of Freedom	16
R square	0.6414
Absolute Sum of Squares	7017
Sy.x	20.94
Number of points	
Analyzed	17

# HCC15.1 Tetramer

log(inhibitor) vs. normalized response -- Variable slope

Best-fit values	
LogIC50	-11.15
HillSlope	-0.3465
IC50	7.086E-12
Std. Error	
LogIC50	0.1964
HillSlope	0.06073
95% Confidence Intervals	
LogIC50	-11.57 to -10.73
HillSlope	-0.4777 to -0.2153
IC50	2.668e-012 to 1.882e-011
Goodness of Fit	
Degrees of Freedom	13
R square	0.8525
Absolute Sum of Squares	1220
Sy.x	9.686
Number of points	
Analyzed	15

#### HCC15.2 Monomer

	Normalized Binding
log(inhibitor) vs. normalized re	esponse
Best-fit values	
LogIC50	-7.36
IC50	4.362E-08
Std. Error	
LogIC50	0.1714
95% Confidence Intervals	
LogIC50	-7.737 to -6.983
IC50	1.830e-008 to 1.040e-007
Goodness of Fit	
Degrees of Freedom	11
R square	0.7055
Absolute Sum of Squares	3564
Sy.x	18
Number of points	
Analyzed	12







## HCC15.2 Tetramer

log(inhibitor) vs. normalized	response	
Best-fit values		
LogIC50		-7.795
IC50		1.603E-08
Std. Error		
LogIC50		0.1056
95% Confidence Intervals		
LogIC50	-8.014 to -7.576	
IC50	9.680e-009 to 2.654e-008	
Goodness of Fit		
Degrees of Freedom		22
R square		0.8753
Absolute Sum of Squares		3597
Sy.x		12.79
Number of points		
Analyzed		23



# Table S-IV peptide Cross-Blocking Data

		10-Fe	old Excess Unlabled Pep	otide
AF488 Labled Peptide		15.1	15.2	1299.2
	15.1	0.28	0.7	0.56
	15.2	0.12	0.14	0.12
	1299.2	0.15	0.93	0.11

All Data normalized to no competitor peptide

Table S-V Mutational Status of Differen	t Cell Lines											
	EGFR	BRAF	KRAS	NRAS	STK11 (LBK1)	CDKN2A	p53	PTEN	PIK3CA	RB1	Cluster	Phenotype
Calu-3 (lung AD)					, , , , , , , , , , , , , , , , , , ,		1					1 E
11648 (lung AD, lymph node met)						1	1				1	1 E
11993 (lung AD)					1		1				1	1 E
12009 (lung AD, lymph node met)			1	1	1		1			1		1 E
12073 (lung AD)					1		1			1		1 <b>M</b>
PC-9 (lung AD)		1				1	1				1	1 E
1358 (Lung AD/BAC)			-	1			1					1 F
(24Rg / 12/2/10)		1										
calu-1 (lung SQ)			-	1			1					2 M
1299 (lung I C)		1		1								2 M
1355 (lung AD)		1	1	1	1		1					2 M
157 (lung SQ)					1	-	1					2 M
2087 (lung AD lymph node met)		1		1			1					2 M
UH7 (liver)							1		1	1	1	2 ND
DA-MB-231 (breast)		4		1			1	1			1	2 ND
is PoCo (nonorostio)				-	+						1	
iarava (pancreatic)		+		+	+			-			1	
W180 (actor)										+		
	-								+	1		
and r (pandreatic)		1			1						1	
+/D (bleast)												
1975 (lung AD)				+			1					4 E
1819 (lung AD, lymph node met)		+		_			1					4 E
441(lung AD/BAC)		_	1				1					4 E
DA-MB-468 (breast)							1	1	1	1 1		4 ND
549 (lung AD)			1	1	1	1						3 <b>M</b>
1155 (lung LC)			1	1			1	1				3 M
1437 (lung AD)					1	1	1					3 E
1666 (lung AD/BAC)		1			1	1						3 E
2126 (lung AD)					1	1	1					3 <b>E</b>
2882 (lung carcinoma)							1					3 <b>M</b>
460 (lung Ad)			1	1	1	1			1			3 M
CC15 (lung SQ)				1	1	1	1			1		3 M
CC2450 (lung SQ)									1			3 <b>E</b>
CF7(breast)						1			1			3 ND
COV3 (ovarian)						1	1		1			3 ND
lutations are noted in red.												
otal number of samples with mutation	:	2 3	11	1 3	3 11	14	26	4	7	7 4		
ercentage of samples with the mutation	5.71%	6 8.57%	31.43%	6 8.57%	31.43%	40.00%	74.29%	11.43%	20.00%	5 11.43%		
otal Number of Samples: 35												
-												
12009 1 (12)		<b>2</b> 0		3 0	) ?	6	. 11	4		2 °		
2003.1 (12)		<u> </u>	-		, 3		11	1		, J		
1299.2 (20)		J 2	1	/ 2	2 5	5	16	2	. 1	1 2		
CC15.1 (20)	(	) 2	7	7 2	2 5	5	16	2	1	1 2		
CC15.2 (20)	(	) 2	7	7 2	2 5	5	i 16	2	1	1 2		
o Binders (11)	(	D 1	3	3 1	6	9	6	1	4	1 1		
Sec. 1 ( )			,					~				
roup 1 (++++)		i 0	2	<u> </u>	3	2	7	0	(	) 2		
iroup 2 (-+++)	(	0 2	5	5 2	2 2	3	9	2	1	I 0		
3roup 3 ()		1 0	1	1 C	) 0	1	4	1	2	2 1		

#### Table S-VI Statistical Analysis of Correlations between Mutational Status and peptide Binding

2X2 Tables and Fisher's Exact Tests

< H2009>	< H1299.2, HCC15.1, HCC15.2>	Cluster 2	Cluster 1	Cluster 3
H2009 p-value   0 1   EGFR WT 23 10   Mutant 0 2 0.1109	triplet p-value   0 1 p-value   EGFR WT 18 19   Mutant 1 1 1.0000	cluster 2 p-value   0 1   EGFR WT 25 12   Mutant 2 0 0.5956	group 1 p-value   0 1 p-value   EGFR WT 30 7   Mutant 1 1 0.3725	Null p-value   0 1 p-value   EGFR WT 25 12 1.0000   Mutant 2 0 1.0000 1.0000
H2009 p-value   0 1   BRAF WT 20 12   Mutant 3 0 0.5361	triplet p-value   0 1 p-value   BRAF WT 18 18   Mutant 1 2 0.6071	cluster 2 p-value   0 1   BRAF WT 26 10   Mutant 1 2 0.2191	group 1 p-value   0 1 </th <th>Null p-value   0 1 p-value   BRAF WT 25 11 1.0000   Mutant 2 1 1.0000 1.0000</th>	Null p-value   0 1 p-value   BRAF WT 25 11 1.0000   Mutant 2 1 1.0000 1.0000
H2009   0 1 p-value   KRAS WT 15 9 0.7091   Mutant 8 3 0.7091	triplet p-value   0 1 p-value   KRAS WT 15 13   Mutant 4 7 0.4801	cluster 2 p-value   KRAS WT 21 7 0.2681   Mutant 6 5 0.2681	group 1 p-value   0 1 p-value   KRAS WT 22 6   Mutant 9 2 1.0000	Null p-value   0 1   KRAS WT 19 9   Mutant 8 3 1.0000
H2009 p-value   0 1 p-value   NRAS WT 20 12   Mutant 3 0 0.5361	triplet p-value   NRAS WT 18 18 0.6071   Mutant 1 2 0.6071	Image: cluster 2 p-value   0 1 p-value   NRAS WT 26 10   Mutant 1 2 0.2191	group 1 p-value   NRAS WT 28 8 1.0000   Mutant 3 0 1.0000 1.0000	Null p-value   0 1   NRAS WT 25 11   Mutant 2 1 1.0000
H2009 p-value   0 1 p-value   STK11 WT 15 9   Mutant 8 3 0.7091	triplet p-value   0 1 p-value   STK11 WT 13 15   Mutant 6 5 0.7311	Cluster 2 p-value   0 1   STK11 WT 18 10   Mutant 9 2 0.4463	group1 p-value   0 1 p-value   STK11 WT 23 5   Mutant 8 3 0.6632	Null p-value   0 1   STK11 WT 22 6   Mutant 5 6 0.0615
H2009 p-value   0 1 p-value   CDKN2A WT 15 6   Mutant 8 6 0.4769	triplet p-value   0 1 p-value   CDKN2A WT 6 15 0.0104   Mutant 13 5 0.0104	Cluster 2 p-value   0 1 p-value   CDKN2A WT 12 9   Mutant 15 3 0.0959	group 1 p-value   0 1 p-value   CDKN2A WT 15 6   Mutant 16 2 0.2472	Null p-value   0 1 1   CDKN24 WT 18 3 0.0346   Mutant 9 9 0.0346
H2009 p-value   0 1 p-value   p53 WT 8 1   Mutant 15 11 0.1211	triplet p-value   0 1 p-value   p53 WT 5 4   Mutant 14 16 0.1871	Use Cluster 2 p-value   0 1 p-value   p53 WT 6 3   Mutant 21 9 1.0000	group 1 p-value   0 1 1 0 1 0 1 0 1 0 1 0 1 0 1 1 0 0 6526 0 6526 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 <th>Null p-value   0 1   p53 WT 4 5   Mutant 23 7 0.1018</th>	Null p-value   0 1   p53 WT 4 5   Mutant 23 7 0.1018
H2009 p-value   0 1   PTEN WT 20 11   Mutant 3 1 1.0000	triplet p-value   0 1 p-value   PTEN WT 17 18   Mutant 2 2 1.0000	Cluster 2 p-value   0 1 p-value   PTEN WT 25 10   Mutant 2 2 0.5733	group 1 p-value   O 1 p-value   PTEN WT 27 8 0.5628   Mutant 4 0 0.5628	Null p-value   0 1   PTEN WT 24 11   Mutant 3 1 1.0000
H2009 p-value   0 1 p-value   PIK3CA WT 18 10   Mutant 5 2 1.0000	triplet p-value   0 1 1   PIK3CA WT 10 19 0.0033   Mutant 9 1 0 0	Cluster 2 p-value   0 1 p-value   PIK3CA WT 20 11   Mutant 7 1 0.3938	group 1 p-value   0 1 p-value   PIK3CA WT 23 8   Mutant 8 0 0.1683	Null p-value   0 1 1   PIK3CA WT 23 7 0.1038   Mutant 4 5 0.1038
H2009 p-value   0 1 p-value   RB1 WT 22 9   Mutant 1 3 0.1061	triplet p-value   0 1 p-value   RB1 WT 17 18   Mutant 2 2 1.0000	cluster 2 p-value   0 1 p-value   RB1 WT 23 12   Mutant 4 0 0.2916	group 1 p-value   0 1 p-value   RB1 WT 29 6   Mutant 2 2 0.1803	Null p-value   0 1   RB1 WT 24 11   Mutant 3 1 1.0000
H2009 p-value   KRAS btherwise 19 11 0.6399   STK11 2 X Mut 4 1 0.6399	H1299 p-value   KRAS otherwise 13 17   STK11 2 X Mut 2 3	Null Group p-value   KRAS otherwise 21 9 0.6399   STK11 2 X Mut 3 2 0.6399		
H2009 p-value   0 1   KRAS otherwise 18 12   CDKN2 2 X Mut 5 0 0.1412	H1299 p-value   0 1   KRAS otherwise   13 17   CDKN2 2 X Mut   2 3	Null Group p-value   0 1 p-value   KRAS otherwise 21 9   CDKN2 2 X Mut 3 2		
H2009 p-value   0 1 p-value   STK11 btherwise 16 12 0.0695   CDKN2 2 X Mut 7 0 0	H1299 p-value   0 1   STK11 otherwise 9 19 0.0274   CDKN2 2 X Mut 6 1 1	Null Group p-value   0 1 p-value   STK11 btherwise 23 5 0.0017   CDKN2 2 X Mut 1 6 1		

#### Table S-VII RPPA Correlation Data

H2000 1 Analysis	Table 3-VII KFFA Collelation	Data		
Marker	ttest p value	FullSerum High.vs.Low.FoldChange	Pearson Correlation	Pearson P value
pMet_Tyr1234/1235	0.000831888	26.22884228	0.581864805	0.004500447
ATR	0.013944487	8.144478052	0.570587521	0.00555347
E Cadherin	0.001049363	4.03952645	0.583287481	0.004380326
Met	0.007218458	3.755117416	0.544074815	0.008854727
STAT5_pY694	0.0003967	3.142244909	0.714167552	0.000189006
EGFR_pY1173	0.002363457	3.139161137	0.580030013	0.004659411
pAxl Y779	0.012085971	2.914584473	0.560394064	0.006674249
STAT6_pY641	0.007460998	2.728949329	0.551371743	0.00781674
beta-Catenin	0.005461583	2.336955152	0.446872546	0.037066428
Her2_pY1248	0.001064712	2.208096008	0.606850653	0.002747734
MACC1	0.005369179	1.894460728	0.462996203	0.030012015
Src_pY416	0.02574993	1.80260343	0.48998129	0.020621459
STAT3_pY705-2	0.015883992	1.757080299	0.529403496	0.011286194
STAT3_pY705-1	0.041953767	1.618057612	0.550791596	0.007895406
N Cadherin	0.001851895	-1.168381738	-0.658269446	0.000866922
TAZ.1	0.028214489	-1.180086868	-0.548418902	0.008223966
Stathamin	0.03220062	-1.424910793	-0.377902069	0.082913829
RAD50	0.028171093	-1.596260352	-0.470027588	0.027290357
Thymidylate synthase	0.017338785	-1.644269789	-0.49379446	0.019510745
H1299.2/HCC15.1/HCC15.2 Analysis				
Marker	FullSerum ttestpvalue	FullSerum High.vs.Low.FoldChange	Pearson Correlation	Pearson P value
p16	0.013559309	2.031347507	0.382171631	0.079217822
XIAP	0.020023271	-1.185240268	-0.549366751	0.008091386
VASP	0.004237199	1.429702674	0.415426389	0.054516309
PDK1_pS241	0.047194833	-1.230937203	-0.366630969	0.093288979
Caspase 7	0.013390989	-1.323391571	-0.577621644	0.004875154

Markers with p values < 0.05 are included in the table p values < 0.01 are highlighted in yellow

			Table S-VIII NIR	Imaging Raw I	Data									
							H2009 tumor/ H460							
H2009.1		2009 tumor	460 tumor	liver	lung	spleen	tumor	H2009 tumor/liver	H2009 tumor/lung	H2009 tumor/spleen	H460/liver	H460/lung	H460/spleen	
30 µg	Mouse 1	181	D 3.	7 780	D 75	5 50.20	49.5	5 0.2	24.0	36.1	0.005	i	0.49	0.73
30 µg	Mouse 2	1730	0 42	3 1050	0 2270	Below detection lim	40.4	l 1.6	5 7.6	6	0.041		0.19	
30 µg	Mouse 3	930	0 43	3 758	D 48	3 26.20	21.5	5 1.2	192.9	355.0	0.057	,	8.98	16.53
30 µg	Mouse 4	797	0 38-	1 559	0 17	7 2.13	20.8	3 1.4	45.0	3741.8	0.069	1	2.17	180.28
30 µg	Mouse 5	1360	D 11:	2 931	D 129	9 18.30	121.4	l 1.5	5 105.4	4 743.2	0.012	<u>:</u>	0.87	6.12
	average	999	6 27	815	6 540	24.21	50.7	1.2	2 75.0	) 1219.0	0.037	,	2.54	50.91
	SEM	262	6 8	5 83	3 433	3 10	19	0.25	i 34	4 853	0.012	1	1.65	43.25
							H1299 tumor/ H460							
H1299.2		1299 tumor	460 tumor	liver	lung	spleen	tumor	H1299 tumor/liver	H1299 tumor/lung	H2009 tumor/spleen	H460/liver	H460/lung	H460/spleen	
53 µg	Mouse 1	820	0 416	5 4	1 3050	below detection	2.0	) 200.2	2.7	7	101.71		1.37	
53 µg	Mouse 2	235	5 61	3 310.7	0 4685	5 84.60	3.8	3 7.6	6 0.5	5 27.8	1.99	1	0.13	7.30
53 µg	Mouse 3	971	0 207	0 65	2 1475	5 146	4.7	14.9	6.6	66.7	3.18	i	1.40	14.23
	average	675	5 228	4 33	4 3070	) 115.05	3.5	5 74.2	3.3	3 47.3	35.62	1	0.97	10.76
	SEM	2242.7	7 1783.43	2 305.9	6 926.70	30.45	0.80	) 63.04	1.78	3	33.04	<u> </u>	0.42	
							H1299 tumor/ H460							
15.1		1299 tumor	460 tumor	liver	lung	spleen	tumor	H 1299 tumor/liver	H1299 tumor/lung	H1299 tumor/spieen	H460/Liver	H460/Lung	H460/spleen	
43 µg	wouse 1	2410	J 50.	3 657	J 432	2 12.64	4.8	3 0.4	5.0	5 190.7	0.08		1.16	39.81
43 µg	wouse 2	203	J 57.	2 402	5 2/0	below detection	3.6	0.5	) /.:		0.14		2.12	
63 ug	Mouse 3	101:	3 68	3 243	5 74'	68.20	1.5	0.4	1.4	14.9	0.28	-	0.93	10.08
	average	181	5 58	434	3 2//.6		3.0	3 0.4	4.8	3 102.8	0.17		1.40	24.95
	SEIVI	417.0	5 53.8	5 1204.2	4 138.20	3	0.97	0.04	1.82	2	0.06		0.36	
1460 1							H460 LUMOI/ H1299	HACO human /liver	UACO human/lung	HACO tumor (anloon	H1200/Liver	L11 200 /luma	111200/emlean	
AA ug	Mouse 1	16	a 142	5 1/8	n a	1 16.0	8/	1	15 1	1 80.3	0.11	HIZ55/Tung	1 70	10.60
AA 110	Mouse 2	no tumor	160	5 124	5 04	64.6	0.4		24.0	24.0	0.11			10.00
89 110	Mouse 3	10	5 486	5 338	n 1944	5 63.9	46.3	14	21.0	5 761	0.03	i.	0.05	1 64
05 45	average	13	7 263	2 203	5 713	2 48.1	27.4	. 19	. 14.3	63.4	0.07	,	0.92	6.12
	SEM	10	1117.8	7 675.9	1 616.64	5 16.09	21.1		64	7 19.66	0.07		0.02	0.12
	J		1117.0	075.5	. 010.0.	10.03		0.14	. 0.47	13.00				
							H1299 tumor/ H460							
15.2							tumor	tumor/liver	tumor/lung	tumor/spleen				

1.5

0.4

1.4

14.9

33 µg Mouse 1

1013

688 2435

741

68.20