Supplemental Legend:

Supplemental Figure 1. A comparison of model subcutaneous pancreatic (AsPC-1) tumor volume in mice over time with saline control, human IgG control, and 2 doses of NPC-1C (n=9 per group). Tumor volume was assessed every 3-4 days in each mouse cohort, with reduction in tumor volume in the NPC-1C treated cohort relative to the control groups. Mice received antibodies or saline on days 5 and 8 (denoted by blue arrow) followed by normal human PBMC on days 6 and 9. All injections were intraperitoneal. Asterisk indicates statistical significance with $p<0.05$ by ANOVA. Experiment performed once. IgG, immunoglobulin G; PBMC, peripheral blood mononuclear cells.

Supplemental Figure 2. Comparative localization of NPC-1C into various tissues in mice, including model subcutaneous pancreatic cancer (CFPAC-1) indexed to blood at 1.000. Localization of the antibody to tumor increased with time, highest at day 6. Experiment performed once. Sm, small; Lrg, large; Intest, intestine.

Supplemental Figure 3. Flow histogram of NPC-1C binding to AsPC1 (pancreatic) or TOV-21G (ovarian) cancer cell lines. Grey line and shading indicate isotype control, red line indicates NPC-1C antibody.

Supplemental Figure 4. Detection of MUC5AC and NPC-1C antigens by flow cytometry in lung A549 and pancreatic CFPAC-1 cancer cell lines. MUC5AC-related, the target antigen of NPC-1C in GI mucosa and colorectal and pancreatic cancer in particular, is a distinct antigen from MUC5AC expressed in lung cancer. MAB2011 (left)

and 45M1 (right) are MUC5AC detecting antibodies. Green indicates detection of MUC5AC and NPC-1C antigens by flow cytometry.

Supplemental Figure 5. Competitive ELISA between MUC5AC from A549 (lung adenocarcinoma) and CFPAC (pancreatic adenocarcinoma) and LS174T (colorectal adenocarcinoma).

Supplemental Figure 6. Grading of immunohistochemical staining by NPC-1C of colonic mucosa. 0= neg; 4+ strongly positive

Supplemental Table 1. Quantification of murine NPC-1C binding to various tumor cell lines by flow cytometry, values representing percentage of cells stained. Two colorectal, one pancreatic, one ovarian, and one breast tumor cell line were assessed for binding by NPC-1C and an isotype control by flow cytometry. Experiment performed once.

Supplemental Table 2. Quantification of staining intensity of NPC-1C in various human tissues (colorectal cancer, normal colorectal, pancreatic cancer, normal pancreatic, and uterine cancer) rated negative (no staining) to +4 (strong). Samples stained at given intensity level as a fraction with associated percentage in parentheses. Experiment performed once.

Supplemental Table 3. A panel of 35 normal, healthy tissues from 3 unrelated donors was used in normal human tissue cross reactivity study for NPC-1C. The results from this study demonstrate NPC-1C positively stains the target cells in the positive control pancreatic and colon carcinoma, but not other normal human tissues including normal lung and stomach at the optimal concentration except weak positive on normal cecum, jejunum and salivary gland. The lower percentage positivity was observed in the study with real normal, normal adjacent to tumor and normal adjacent to other tumors of colon and pancreatic tissue. The typical staining in tumor specificity of NPC-1C IHC is presented. *Real normal: tissues from subjects with non-cancer illness. ** 1 real normal from premature infant. CHTN, Cooperative Human Tissue Network

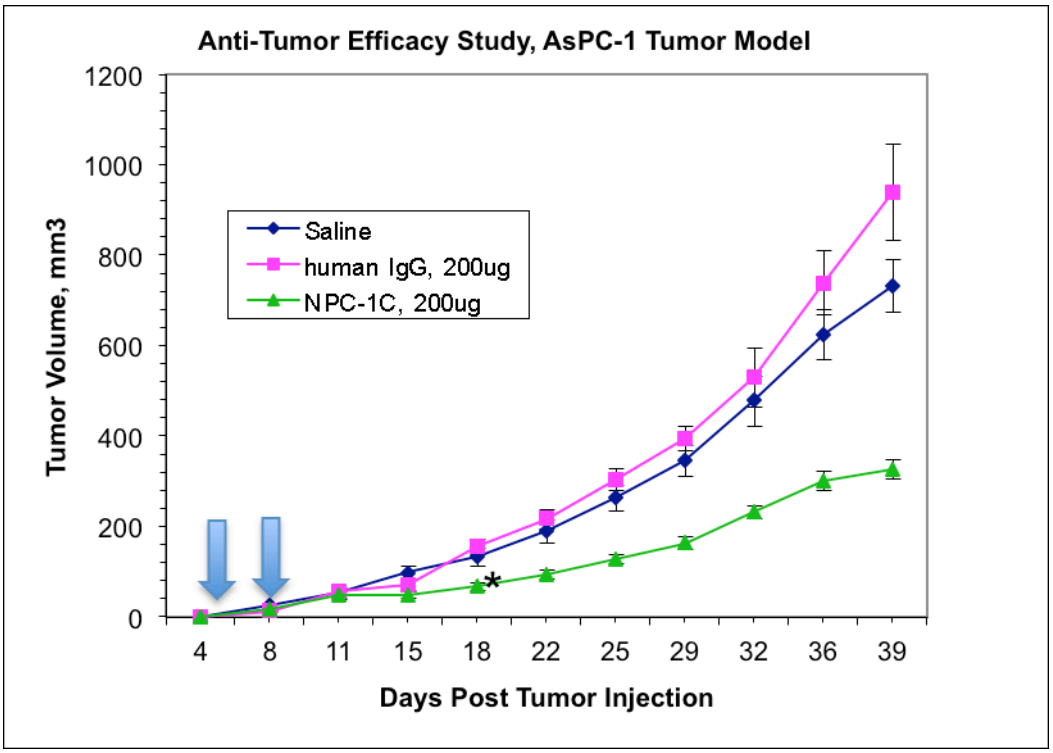
Supplemental Table 4A. Sandwich ELISA with anti-MUC5AC as detection antibody. Values represent optical density at various CFPAC-1 supernatant dilutions. Perlecan represents a control extracellular matrix protein. The table shows increased affinity of the anti-NPC-1C coated plate to MUC5AC versus perlecan controls.

Supplemental Table 4B. Sandwich ELISA with anti-NPC-1C as the detection antibody. Values represent optical density at various CFPAC-1 supernatant dilutions. Perlecan represents a control extracellular matrix protein. The table shows increased affinity of the anti-MUC5AC coated plate for NPC-1C relative to perlecan controls.

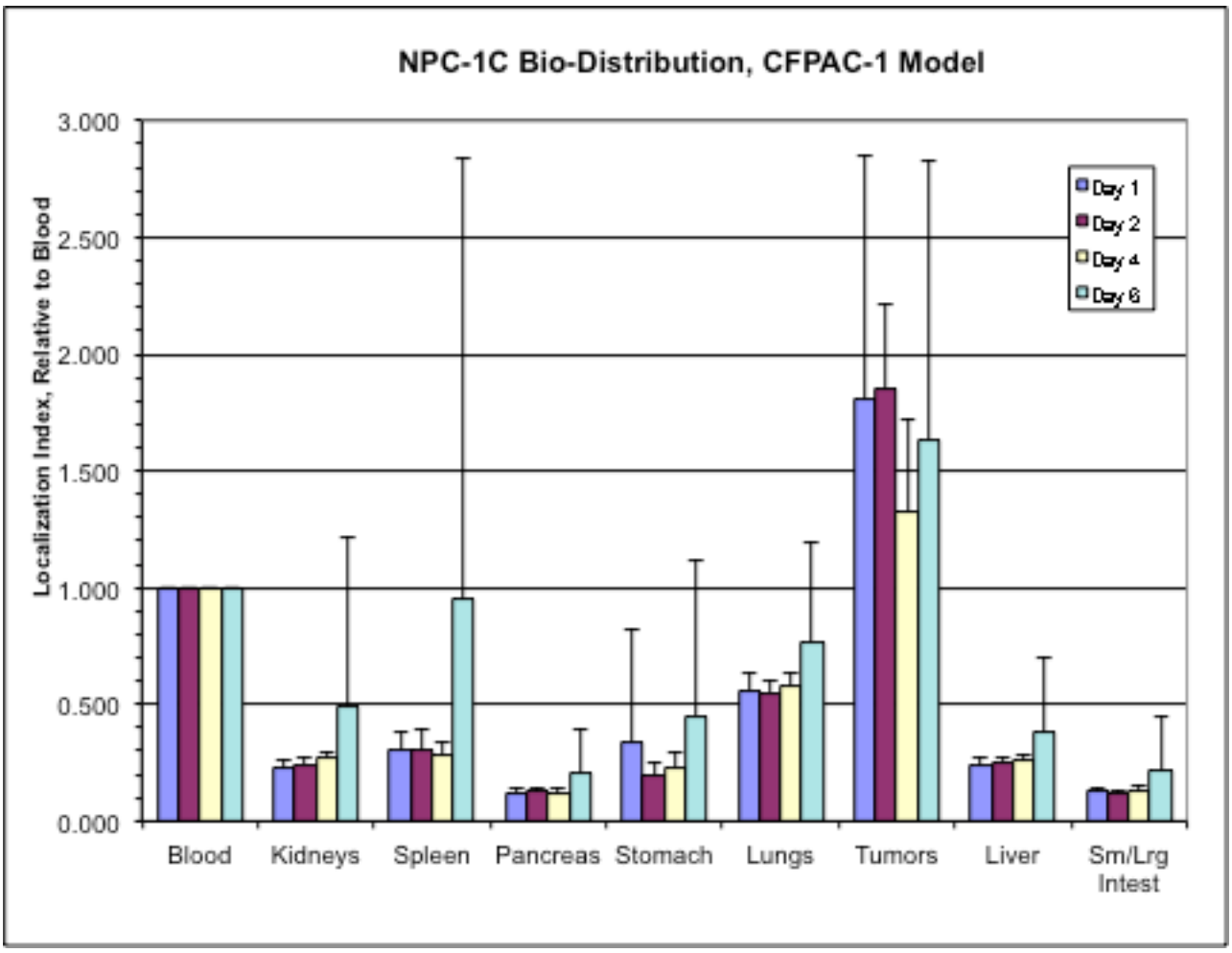
Supplemental Table 5. ADCC by NPC-1C in various tumor cell lines (4 colorectal, 3 pancreatic, 1 melanoma, 1 prostate) based on effector:target ratio. Experiment performed once. ADCC, antibody-dependent cell-mediated cytotoxicity.

Figures:

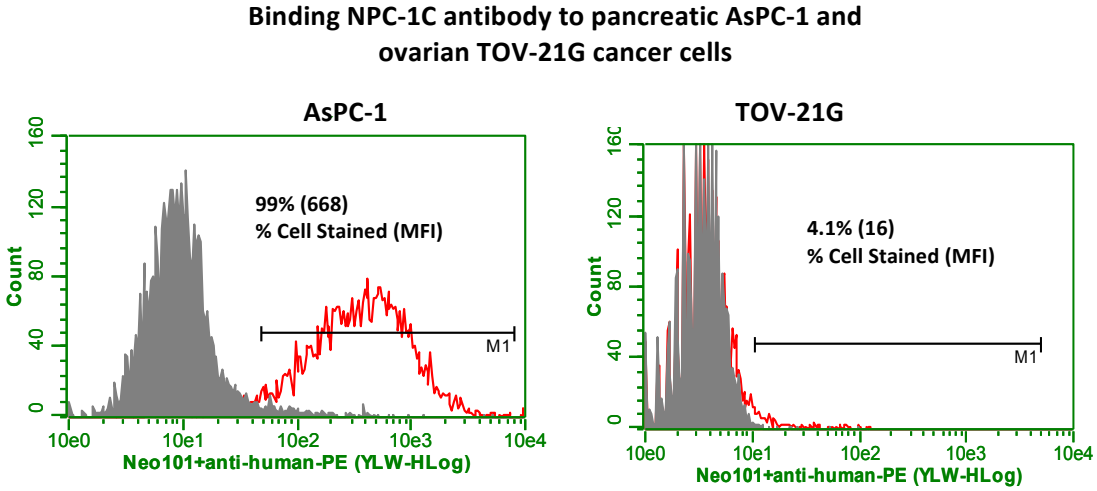
Supplemental Figure 1.



Supplemental Figure 2.



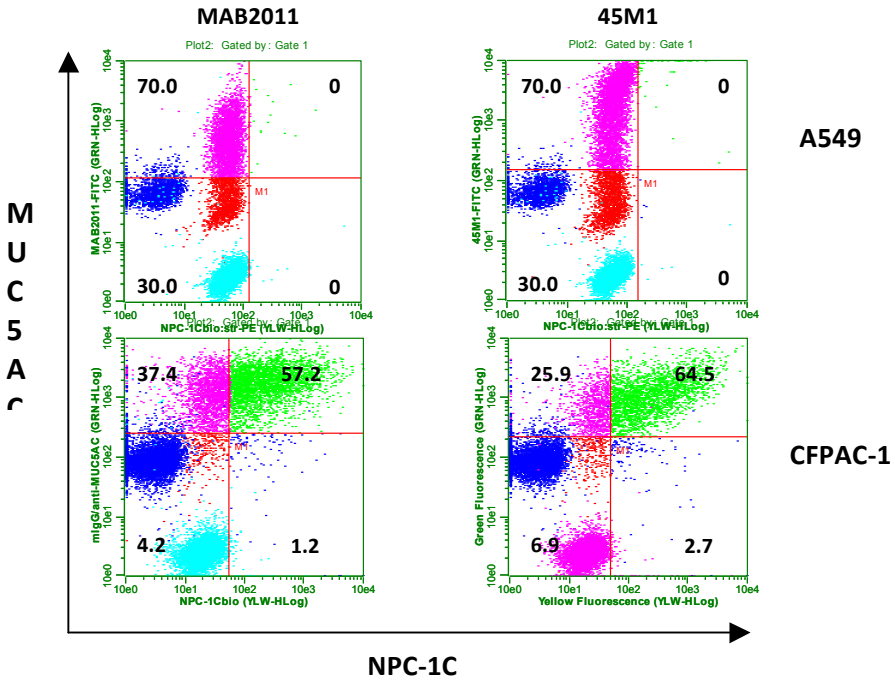
Supplemental Figure 3.



*Isotype control (hIgG)-filled grey histogram;
NPC-1C antibody (20ug/ml)- red line;
%binding and median fluorescent intensity are shown above gate

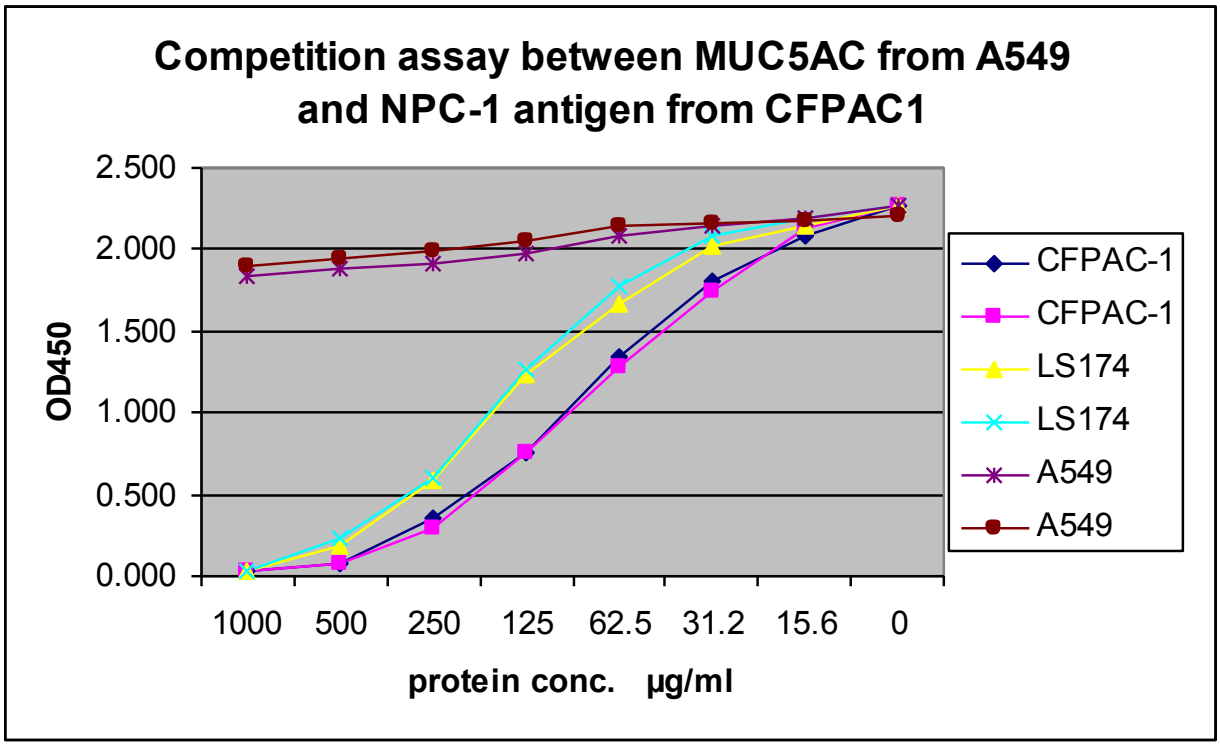
Supplemental Figure 4.

Detection of MUC5AC (45M1 & MAB2011 antibodies) and NPC-1C antigens by flow cytometry in lung A549 and pancreatic CFPAC-1 cancer cells*

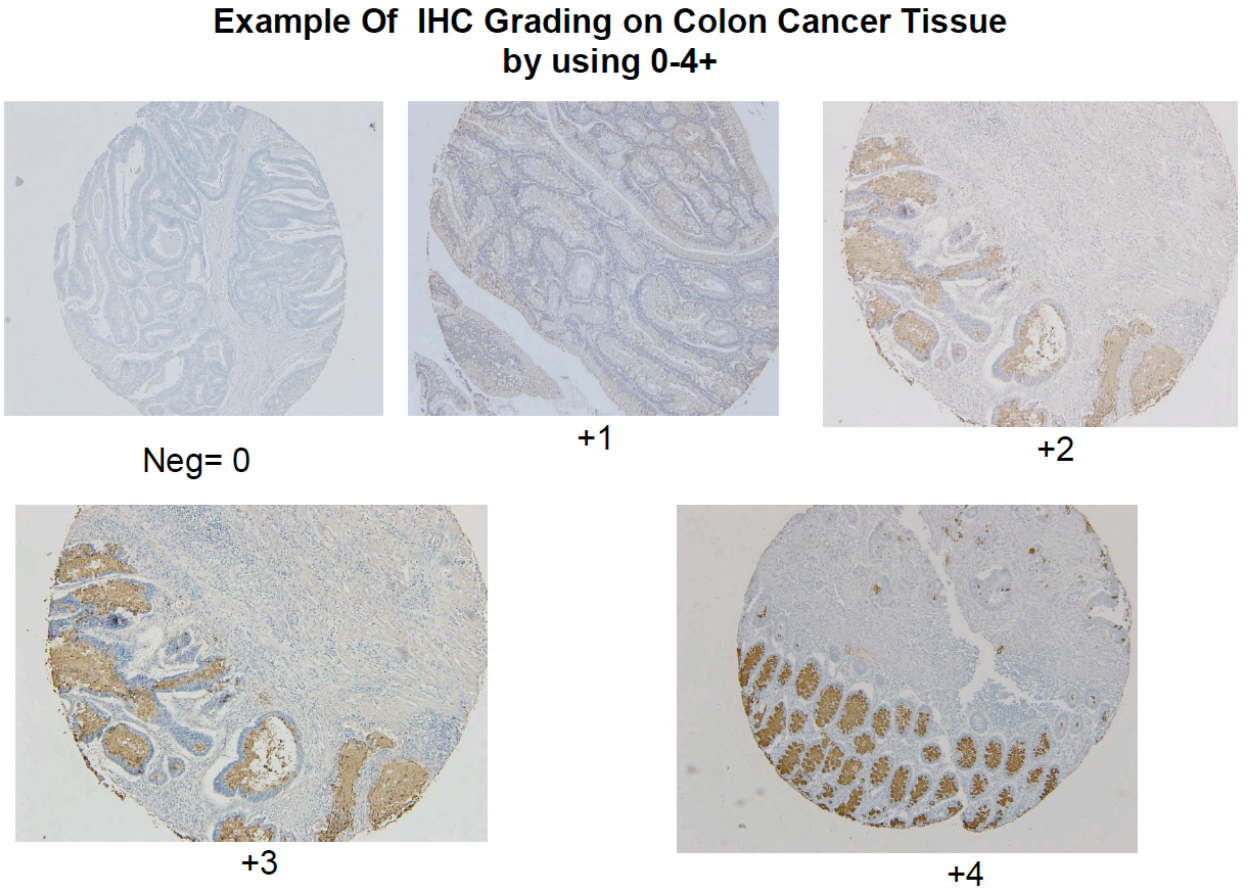


*Overlays of isotype controls which were used to set gates are shown in the left low corner of the dot plots

Supplemental Figure 5.



Supplemental Figure 6.



Tables:

Supplemental Table 1.

Tumor Cell Line	% Cell Staining by FACS	
	Isotype Control	Mouse NPC-1
GEO Colorectal	0.78	86.87
LS174T Colorectal	0.93	57.04
CFPAC-1 Pancreatic	0.44	54.65
OVCAR-3 Ovarian	0.19	0.19
MCF-7 Breast	0.94	0.19

Supplemental Table 2.

Human tissue sample (source)	Tissue staining intensity						
	Negative	Weak	+1	+2	+3	+4	Total Positive
Colon cancer (Accumax array)	27/48 (56%)	5/48 (10%)	7/48 (15%)	4/48 (8%)		5/48 (10%)	21/48 (43%)
Normal colon (Accumax array)	3/4 (75%)			1/4 (25%)			1/4 (25%)
Pancreas cancer (CHTN)	7/11 (64%)		4/11 (32%)				4/11 (32%)
Normal pancreas (CHTN)	3/3 (100%)						0/3 (0%)
Uterus cancer (Accumax array)	32/42 (76%)			2/42 (5%)	8/42 (19%)		10/42 (24%)
Normal uterus (Accumax array)	12/12 (100%)						0/12 (0%)
Prostate cancer (Accumax array)	30/40 (75%)		5/40 (12%)	5/40 (12%)			10/40 (25%)
Normal prostate (Accumax array)	4/4 (100%)						0/4 (0%)

Supplemental Table 3.

		FFPE Tissue	Microarray
Pancreas	Real Normal *	0/17 (0%)	1/46 (2.2%)
	Normal Adjacent to pancreatic Ca	2/10 (20%)	
	Normal Adjacent to other Ca.	1/4 (25%)	
Colon	Real Normal	3/19 (15.8%)**	4/33 (12.1%)
	Normal Adjacent to colon Ca.	2/10 (20%)	
	Normal Adjacent to other Ca.	1/5 (20%)	

Supplemental Table 4A.

CFPAC-1 supernatant Dilution	**Plate coated with NPC-1C antibody (2µg/ml) OD Values**		
1	1.371	0.041	0.041
2	1.046	0.034	0.041
4	0.916	0.027	0.045
8	0.547	0.037	0.057
16	0.337	0.031	0.057
32	0.100	0.020	0.034
64	0.089	0.027	0.034
Detected with	**anti-MUC5AC**	**anti-Perlecan(1)**	**anti-Perlecan (2)**

Supplemental Table 4B.

CFPAC-1 supernatant dilution	**Plate coated with 2µg/ml of either**		
	anti-MUC5AC	**anti-Perlecan(1)**	**anti-Perlecan(2)**
1	2.185	0.166	0.014
2	1.699	0.199	0.025
4	1.996	0.166	0.026
8	1.682	0.124	0.031
16	1.083	0.110	0.029
32	0.645	0.116	0.034
64	0.515	0.081	0.021
Detected with	**NPC-1C**		

Supplemental Table 5.

Tumor Cell Line Target	Effector:Target Cell Ratio	% Specific Killing (± SEM)	
		Isotype control Ab	NPC-1C
Colo-205 (Colorectal)	50:1	9.8 ± 1.9	66.7 ± 0.6
	25:1	0.8 ± 1.2	46.4 ± 1.6
	12.5:1	-0.5 ± 0.1	32.8 ± 2.0
SW620 (Colorectal)	50:1	1.6 ± 0.2	63.7 ± 2.9
	25:1	3.5 ± 1.8	61.0 ± 1.8
	12.5:1	0.0 ± 0.3	51.5 ± 0.9
SW1463 (Colorectal)	50:1	0.1 ± 1.1	33.8 ± 1.0
	25:1	-1.3 ± 0.2	25.5 ± 0.6
	12.5:1	-1.2 ± 0.1	17.9 ± 1.7
LS174T (Colorectal)	50:1	-1.2 ± 0.1	26.8 ± 2.9
	25:1	-0.8 ± 0.1	18.5 ± 4.1
	12.5:1	-1.1 ± 0.0	9.5 ± 0.5
AsPC-1 (Pancreatic)	50:1	-0.8 ± 2.9	44.5 ± 6.8
	25:1	-7.0 ± 2.2	36.2 ± 2.6
	12.5:1	-1.2 ± 0.9	26.5 ± 6.7
CFPAC-1 (Pancreatic)	50:1	-1.2 ± 2.3	26.9 ± 1.6
	25:1	-2.4 ± 0.1	23.2 ± 2.2
	12.5:1	-2.0 ± 0.4	11.1 ± 1.6
PANC-1 (Pancreatic)	50:1	-2.2 ± 0.4	46.8 ± 2.1
	25:1	-2.5 ± 0.4	33.2 ± 3.3
	12.5:1	-3.9 ± 0.3	21.2 ± 0.6
SK-MEL (Melanoma)	50:1	2.7 ± 0.7	4.6 ± 1.1
	25:1	1.5 ± 0.3	3.3 ± 1.1
	12.5:1	1.6 ± 0.4	2.3 ± 0.6
DU145 (Prostate)	50:1	-0.3 ± 0.2	-0.5 ± 0.3
	25:1	-0.7 ± 0.1	0.3 ± 0.8
	12.5:1	-0.2 ± 0.2	-0.3 ± 0.1