(A)

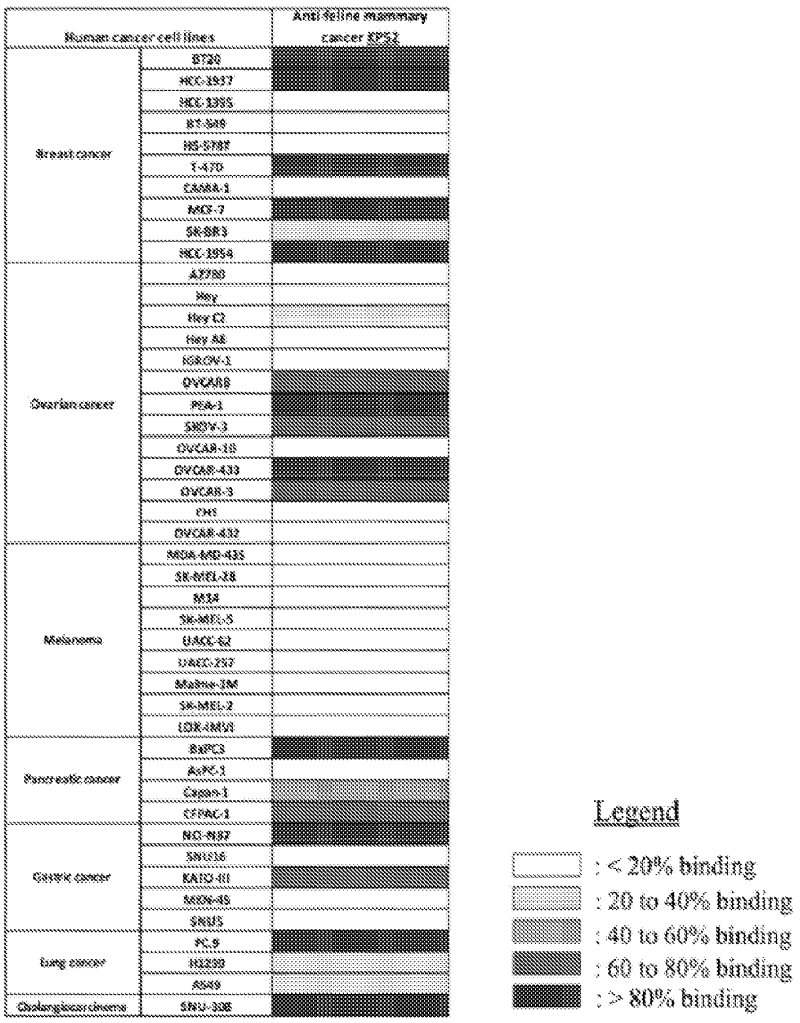


Fig 1(a) (continued)

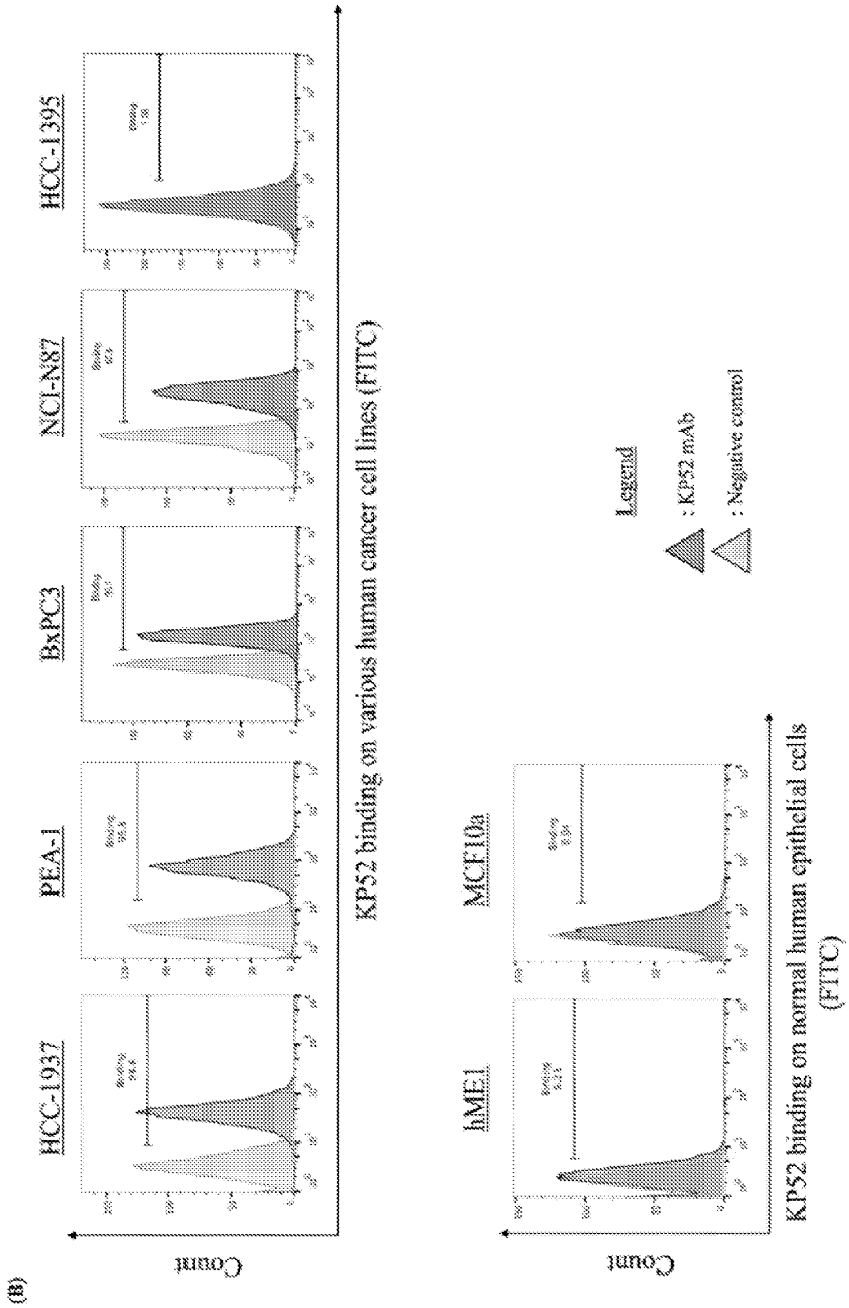


Figure 1(b) (end)

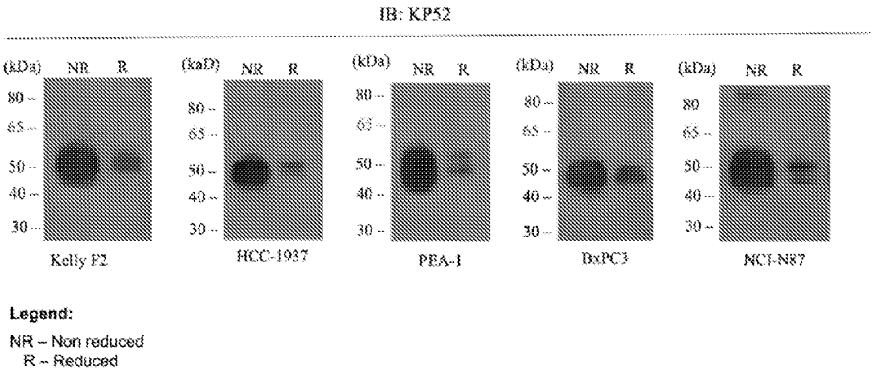

IB: KP52
(kDa)
NR
R
80
65
50
40
30
Kelly F2
(kaD)
HCC-1937
PEA-1
BxPC3
NCI-N87
Legend:
NR – Non reduced
R – Reduced

Figure 2

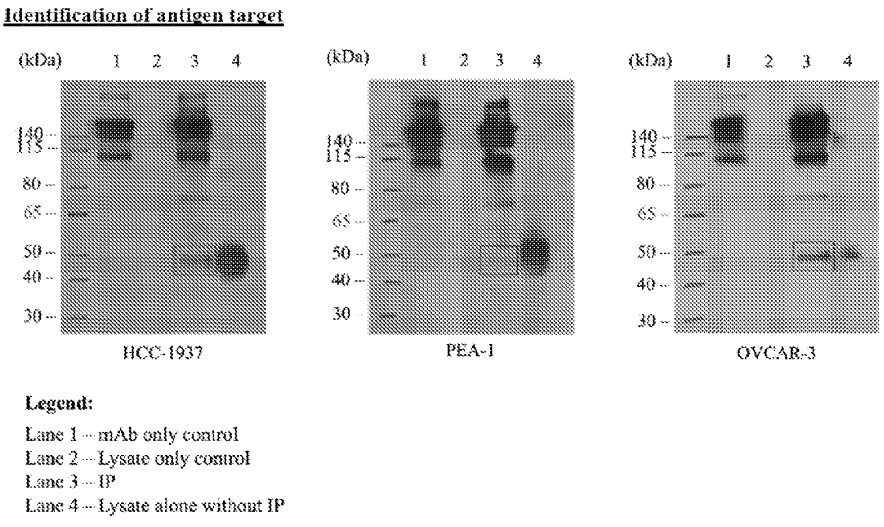

Identification of antigen target
(kDa)
1 2 3 4
140
115
80
65
50
40
30
HCC-1937
PEA-1
OVCAR-3
Legend:
Lane 1 – mAb only control
Lane 2 – Lysate only control
Lane 3 – IP
Lane 4 – Lysate alone without IP

Figure 3

Accession	Description	Coverage	# Peptides	# AAs	MW [kDa]
P09758	Tumour-associated calcium signal transducer 2 OS=Homo sapiens OX=9606 GN=TACSTD2 PE=1 SV=3 - [TACD2_HUMAN]	37.46	9	323	35.7
	Sequence				
	AAGDVDIGDAAYYFER (213) (SEQ ID NO: 7)				
	TLVRPSEHALVDNDGLYDPDcDPEGR (88) (SEQ ID NO: 8)				
	ALGSGmAVDcSTLTSK (57) (SEQ ID NO: 9)				
	ALGSGMAVDcSTLTSK (57) (SEQ ID NO: 10)				
	TLIYYLDEIPPK (256) (SEQ ID NO: 11)				
	MTVcSEGGRGGR (41) (SEQ ID NO: 12)				
	DEKGESLFQGR (229) (SEQ ID NO: 13)				
	FDXGDLSLR (136) (SEQ ID NO: 14)				
	TrmRLEDLR (151) (SEQ ID NO: 15)				
	VRGEPLQVER (248) (SEQ ID NO: 16)				



>sp|P09758|TACD2_HUMAN Tumour-associated calcium signal transducer 2 OS=Homo sapiens OX=9606 GN=TACSTD2 PE=1 SV=3

MARGPGLAPPPLRLPLLLLVLAAVTGHTAAQDNCTCPTNKMTVCSPDGPGGRCQCRALGS
GMAVDCSTLTSKCLLLKARMSAPKNARTLVRPSEHALVDNDGLYDPDCDPEGRFKARQCN
QTSVCWCVNSVGVRRTDKGDLSLRCDELVRTHHILIDLRHRPTAGAFNHSDLDAELRRLF
RERYRLHPKFVAAVHYEQPTIQIELRQNTSQKAAGDVDIGDAAYYFERDIKGESLFQGRG
GLDLRVRGEPLQVERTLIYYLDEIPPKFSMKRLTAGLIAVIVVVVVALVAGMAVLVITNR
RKSCKYKKVEIKELGELRKEPSL(SEQ ID NO: 17)

Figure 4

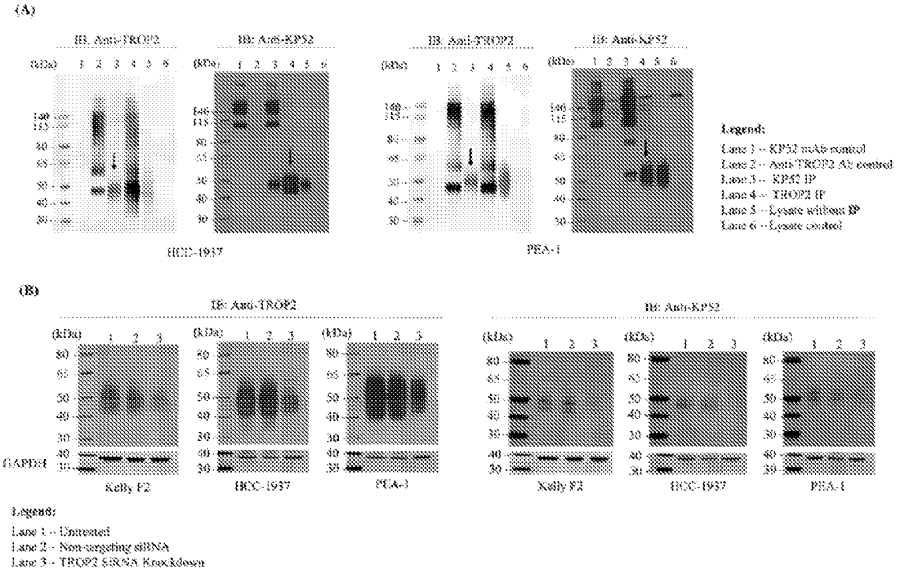

(A)
IB: Anti-TROP2
IB: Anti-KP52
IB: Anti-TROP2
IB: Anti-KP52
HCC-1937
PEA-1
Legend:
Lane 1 – KP52 mAb control
Lane 2 – Anti-TROP2 Ab control
Lane 3 – KP52 IP
Lane 4 – TROP2 IP
Lane 5 – Lysate without IP
Lane 6 – Lysate control
(B)
IB: Anti-TROP2
IB: Anti-KP52
GAPDH
Kelly F2
HCC-1937
PEA-1
Legend:
Lane 1 – Untreated
Lane 2 – Non-targeting siRNA
Lane 3 – TROP2 SIRNA Knockdown

Figure 5

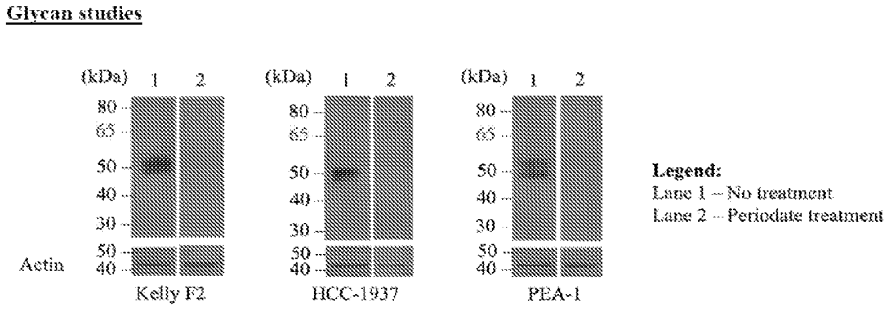

Glycan studies
(kDa)
1
2
80
65
50
40
30
50
40
Actin
Kelly F2
HCC-1937
PEA-1
Legend:
Lane 1 – No treatment
Lane 2 – Periodate treatment

Figure 6

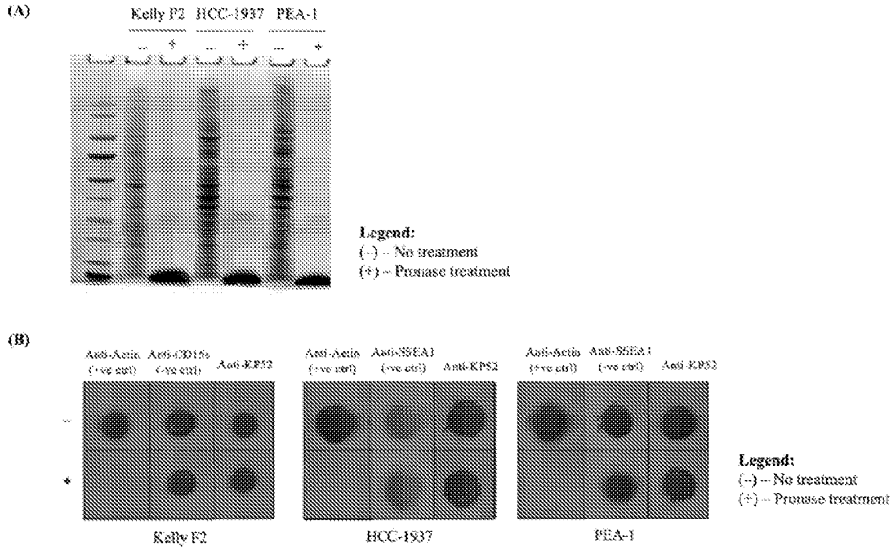
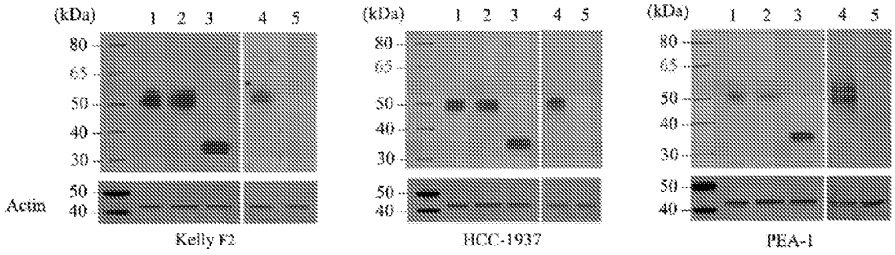

(A)
Kelly F2
HCC-1937
PEA-1
Legend:
(-) – No treatment
(+) – Protease treatment
(B)
Anti-Actin
Anti-KRS2
Kelly F2
HCC-1937
PEA-1
Legend:
(-) – No treatment
(+) – Protease treatment

Figure 7



Legend:
PNGase F assay
Lane 1 – Buffer control
Lane 2 – No treatment
Lane 3 – PNGase F treatment

Beta-elimination assay
Lane 4 – No treatment
Lane 5 – Beta-elimination treatment

Figure 8

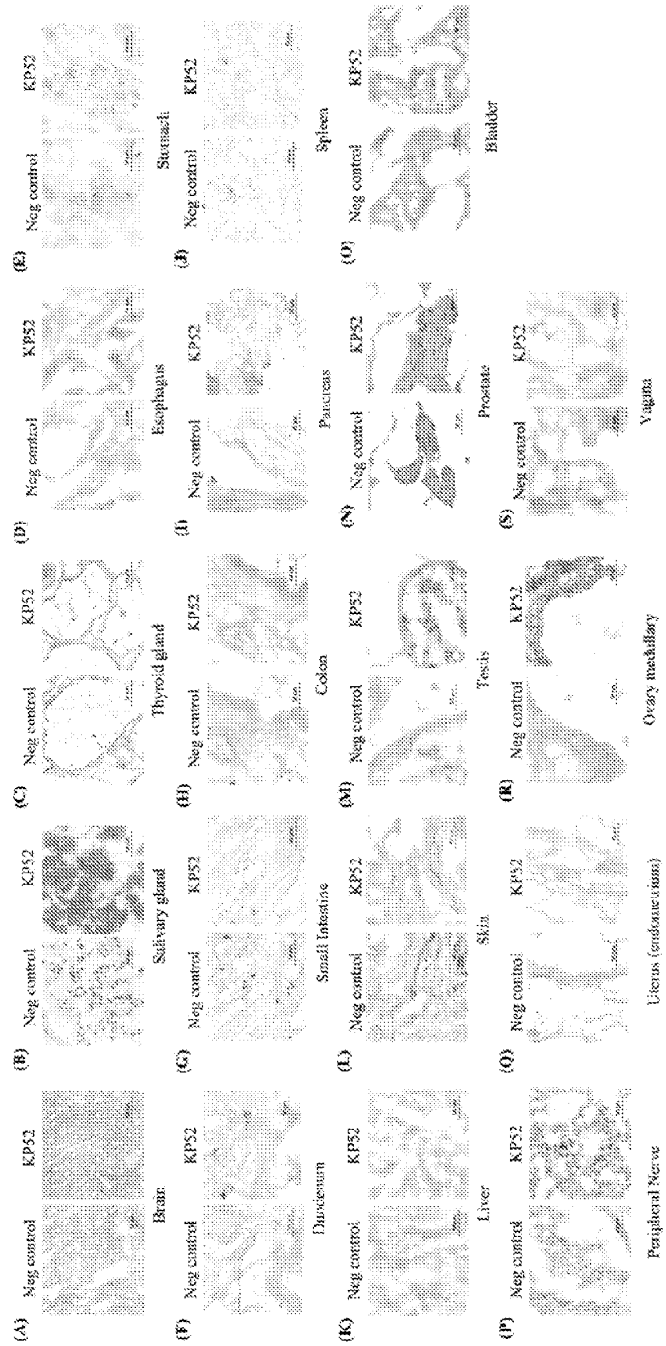

(A)
Neg control
KP52
Brain
(B)
Neg control
KP52
Salivary gland
(C)
Neg control
KP52
Thyroid gland
(D)
Neg control
KP52
Esophagus
(E)
Neg control
KP52
Stomach
(F)
Neg control
KP52
Duodenum
(G)
Neg control
KP52
Small Intestine
(H)
Neg control
KP52
Colon
(I)
Neg control
KP52
Pancreas
(J)
Neg control
KP52
Spleen
(K)
Neg control
KP52
Liver
(L)
Neg control
KP52
Skin
(M)
Neg control
KP52
Testis
(N)
Neg control
KP52
Prostate
(O)
Neg control
KP52
Bladder
(P)
Neg control
KP52
Peripheral Nerve
(Q)
Neg control
KP52
Uterus (endometrium)
(R)
Neg control
KP52
Ovary and fallopian tube
(S)
Neg control
KP52
Vagina

Figure 9

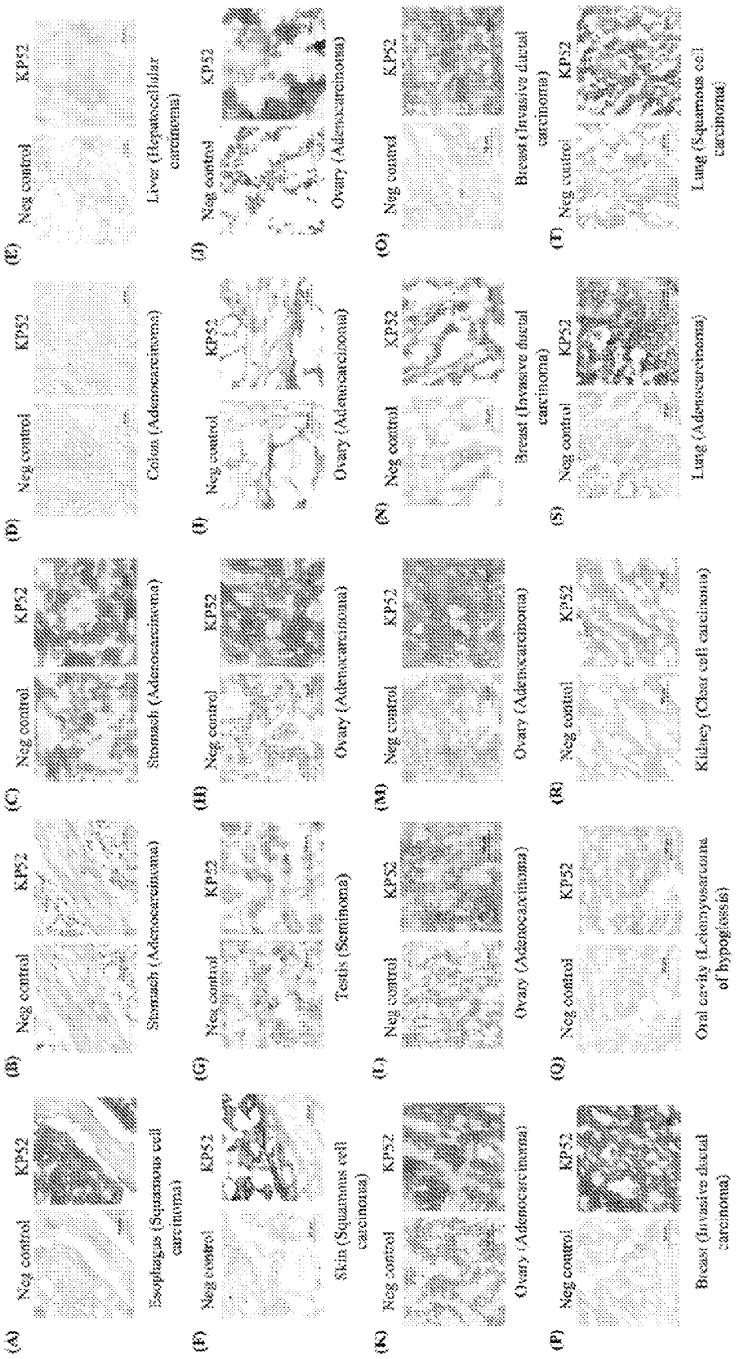

(A)
Neg control
KP52
Esophagus (Squamous cell carcinoma)
(B)
Neg control
KP52
Stomach (Adenocarcinoma)
(C)
Neg control
KP52
Stomach (Adenocarcinoma)
(D)
Neg control
KP52
Colon (Adenocarcinoma)
(E)
Neg control
KP52
Liver (Hepatocellular carcinoma)
(F)
Neg control
KP52
Skin (Squamous cell carcinoma)
(G)
Neg control
KP52
Testis (Seminoma)
(H)
Neg control
KP52
Ovary (Adenocarcinoma)
(I)
Neg control
KP52
Ovary (Adenocarcinoma)
(J)
Neg control
KP52
Ovary (Adenocarcinoma)
(K)
Neg control
KP52
Ovary (Adenocarcinoma)
(L)
Neg control
KP52
Ovary (Adenocarcinoma)
(M)
Neg control
KP52
Ovary (Adenocarcinoma)
(N)
Neg control
KP52
Breast (Invasive ductal carcinoma)
(O)
Neg control
KP52
Breast (Invasive ductal carcinoma)
(P)
Neg control
KP52
Breast (Invasive ductal carcinoma)
(Q)
Neg control
KP52
Oral cavity (Leiomyosarcoma of hypoglossis)
(R)
Neg control
KP52
Kidney (Clear cell carcinoma)
(S)
Neg control
KP52
Lung (Adenocarcinoma)
(T)
Neg control
KP52
Lung (Squamous cell carcinoma)

Figure 10

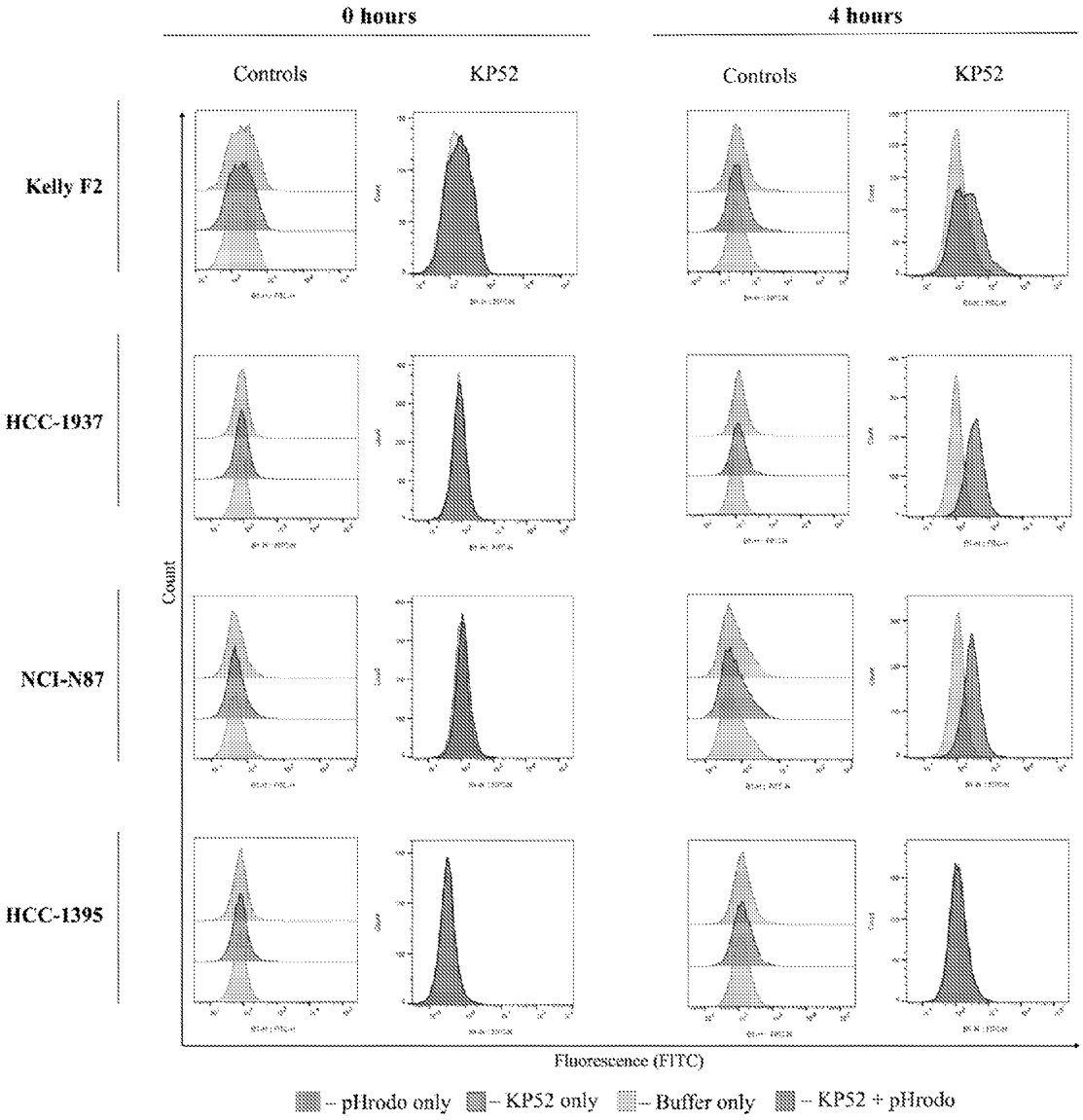

0 hours
4 hours
Controls
KP52
Controls
KP52
Kelly F2
HCC-1937
NCI-N87
HCC-1395
Count
Fluorescence (FITC)
– pHrodo only
– KP52 only
– Buffer only
– KP52 + pHrodo

Figure 11

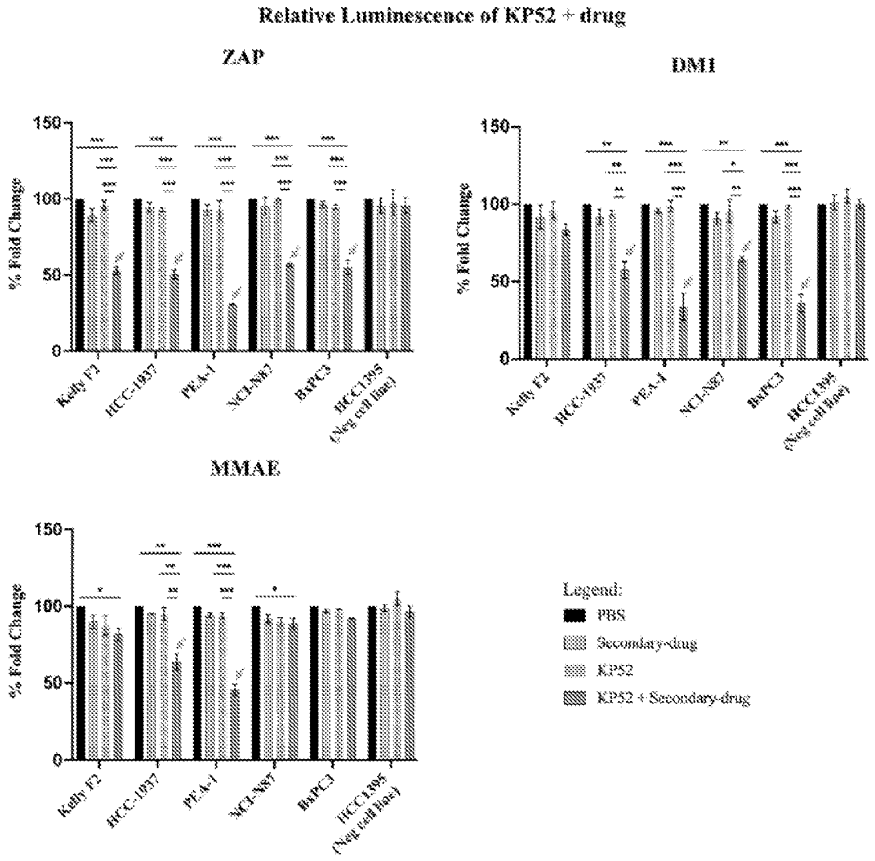

Relative Luminescence of KP52 + drug
ZAP
DM1
MMAE
% Fold Change
150
100
50
0
Kelly F2
HCC-1937
PEA-1
NCI-N87
BxPC3
HCC1395
(Neg cell line)
Legend:
PBS
Secondary-drug
KP52
KP52 + Secondary-drug

Figure 12

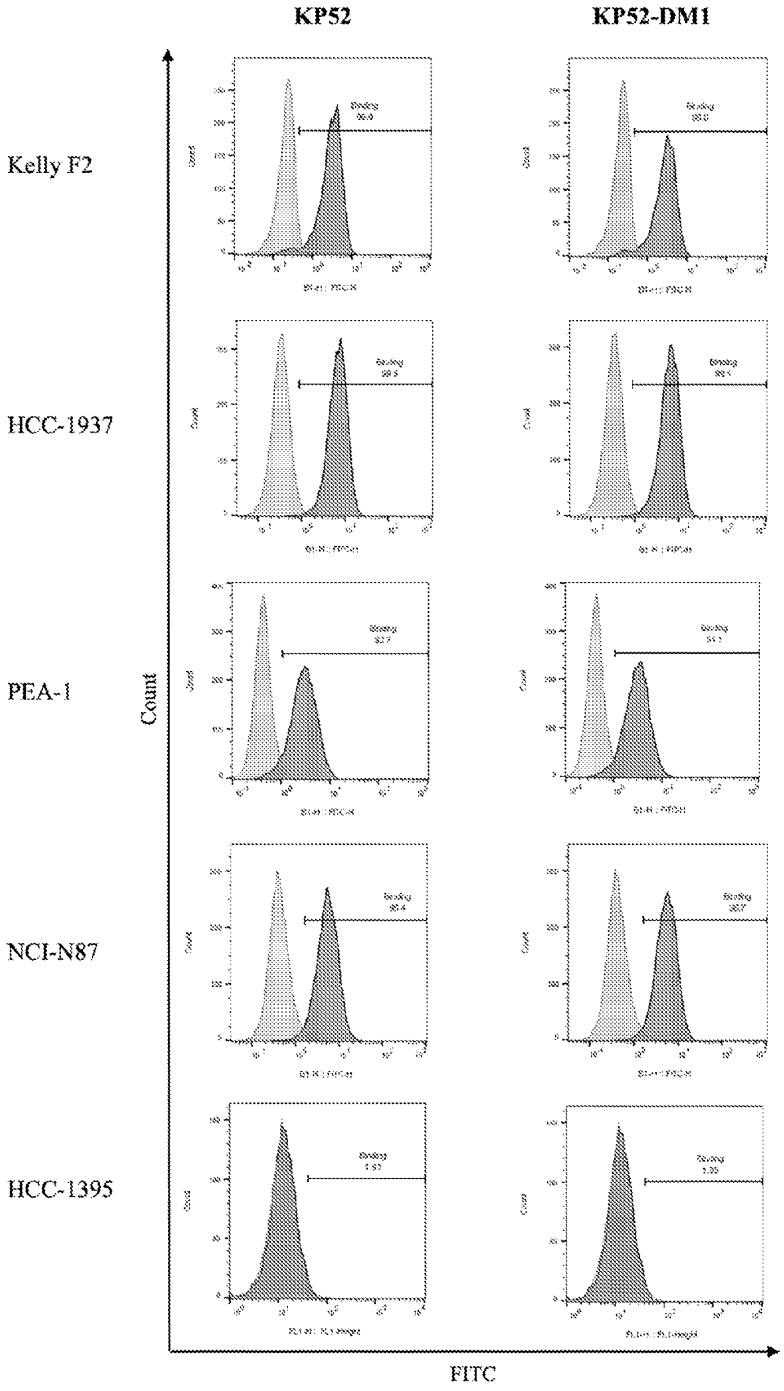

KP52
KP52-DM1
Kelly F2
HCC-1937
PEA-1
NCI-N87
HCC-1395
Count
FITC

Figure 13

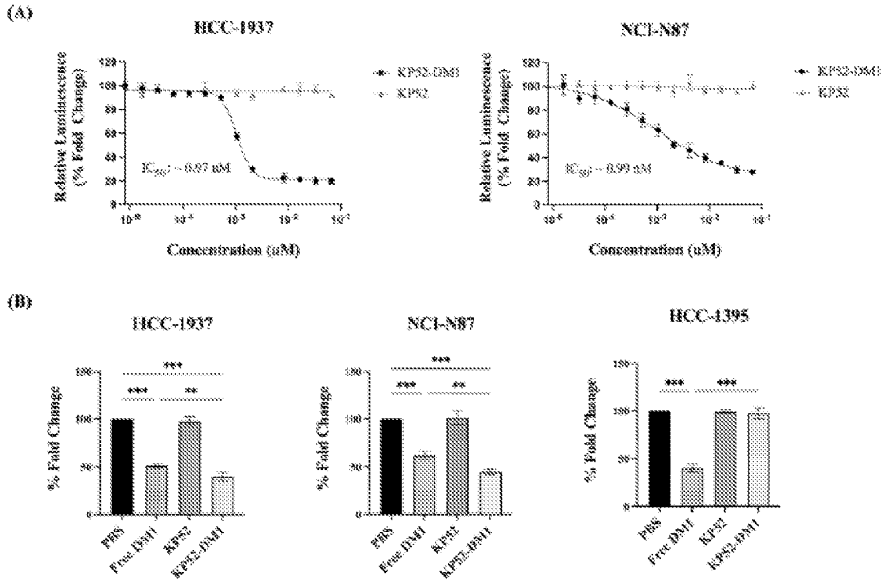

(A)
HCC-1937
NCI-N87
Relative Luminescence (% Fold Change)
Concentration (uM)
KPS2-DM1
KPS2
(B)
HCC-1937
NCI-N87
HCC-1395
% Fold Change
PBS
Free DM1
KPS2
KPS2-DM1

Figure 14

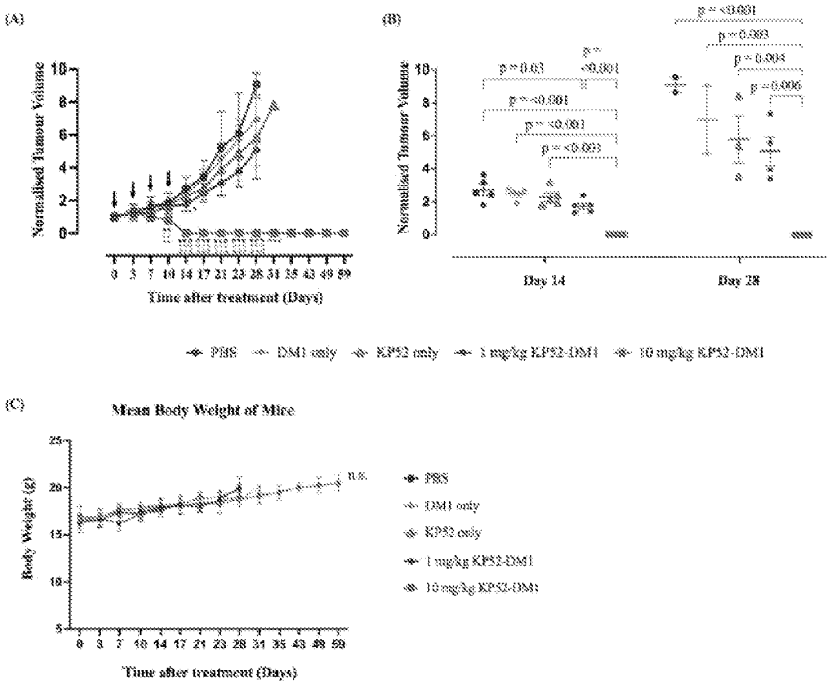


Figure 15(a)-(c) (continued)

D)

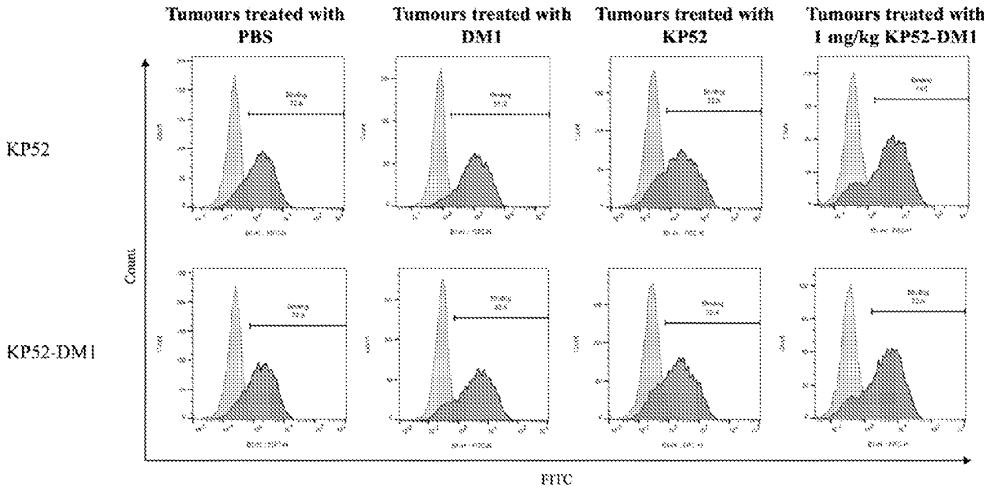


Figure 15(d) (end)

Humanisation of KP52

KP52

	Protein sequence
Heavy chain	QVQLQQSGPELVRPGASVRVSCKASGYSFTDYIITWVKQRTGQGLEWIGQIYPGSGSTYYNEKFKAKATL TADKSSNTAYMHLSSLTSEDSAVYFCSRGIDYDGNWGQGTTLTVSS (SEQ ID NO: 18)
Light chain	DIKMTQSPSSMYASLGERVTITCKASQDINTYLSWFQQKPGKSPKTLIYRANRLVDGVPSRFSGSGSGQD YFLTISSLEDQDMGIYYCLQYDEFPLTFGAGTKLELKR (SEQ ID NO: 19)

hKP52

	Protein sequence
Heavy chain (VH1)	QVQLVQSGAEVKKPGASVKVSCKASGYSFTDYIITWVRQATGQGLEWMGQIYPGSGSTYYNEKFKARVTM TRNTSISTAYMELSSLRSEDTAVYFCARGIDYDGNWGQGTLVTVSS (SEQ ID NO: 20)
Heavy chain (VH2)	QVQLVQSGPEVKRPGASVRVSCKASGYSFTDYIITWVRQAPGQGLEWMGQIYPGSGSTYYNEKFKARVTL TADKSINTAYMELSSLTSEDTAVYFCARGIDYDGNWGQGTLVTVSS (SEQ ID NO: 21)
Light chain (VL1)	DIQMTQSPSSLSASVGDRVTITCKASQDINTYLSWFQQKPGKAPKLLIYRANRLVDGVPSRFSGSGSGTD FTFTISSLQPEDIATYYCLQYDEFPLTFGGGTKVEIK (SEQ ID NO: 22)
Light chain (VL2)	DIQMTQSPSSLSASVGDRVTITCKASQDINTYLSWFQQKPGKAPKTLIYRANRLVDGVPSRFSGSGSGTD FTFTISSLQPEDIATYYCLQYDEFPLTFGQGTKLEIK (SEQ ID NO: 23)

Human IgG1 constant (P01857)
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYP SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPGK (SEQ ID NO: 24)

Human Ig kappa constant (P01834)
RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS TYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 25)

Figure 16

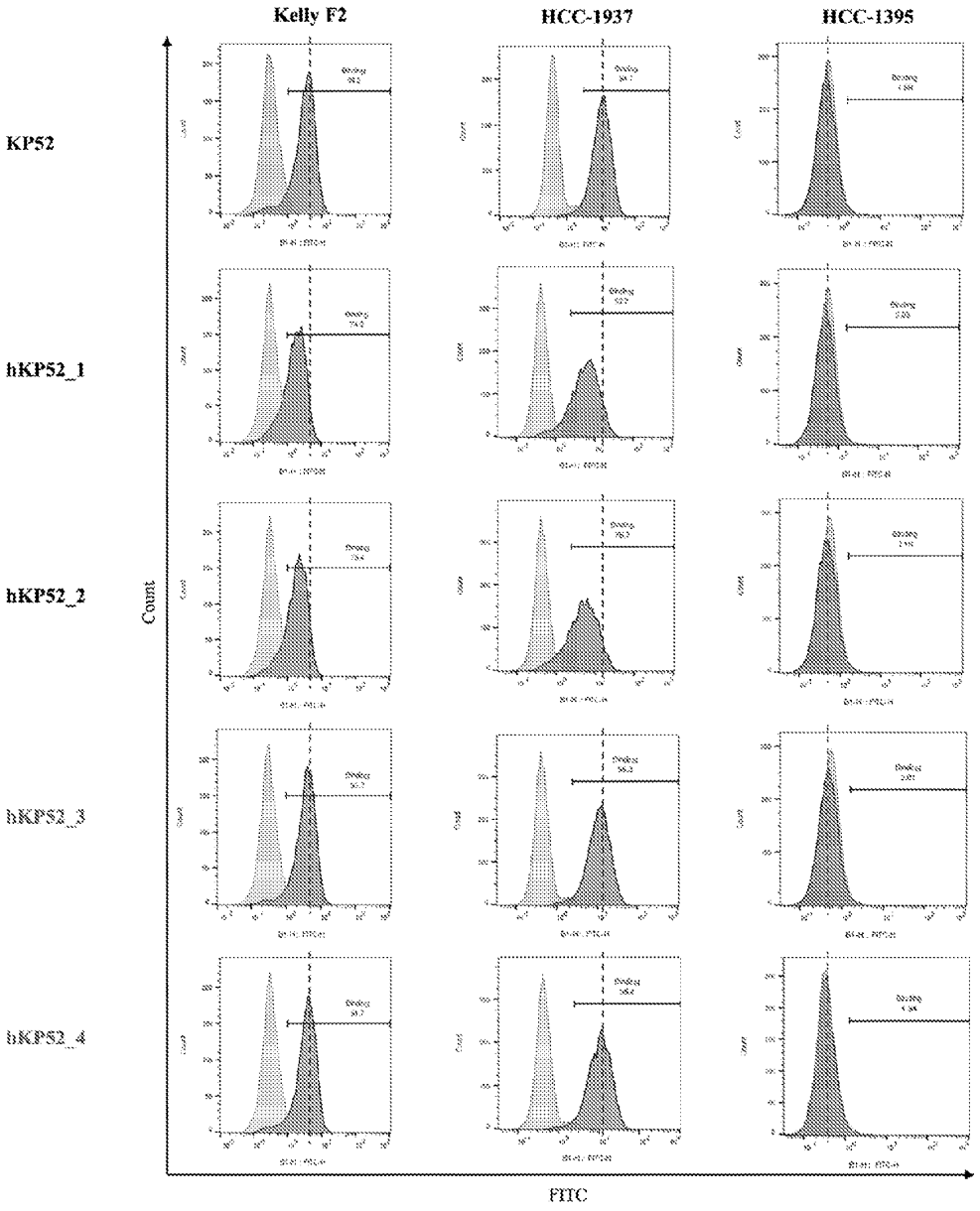

Kelly F2
HCC-1937
HCC-1395
KP52
hKP52_1
hKP52_2
hKP52_3
hKP52_4
Count
FITC

Figure 17

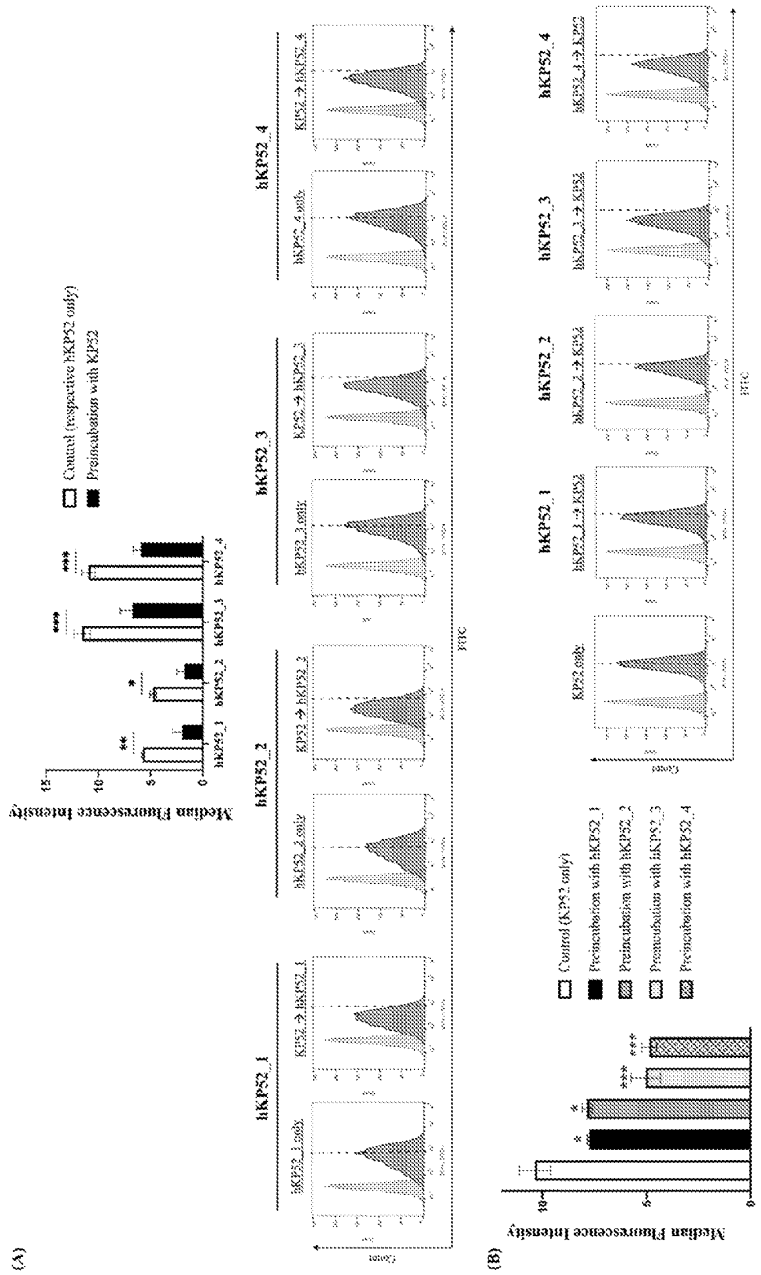

(A)
Median Fluorescence Intensity
Control (respective hKP52 only)
Preincubation with KP52
hKP52_1
hKP52_2
hKP52_3
hKP52_4
(B)
Median Fluorescence Intensity
Control (KP52 only)
Preincubation with hKP52_1
Preincubation with hKP52_2
Preincubation with hKP52_3
Preincubation with hKP52_4

Figure 18

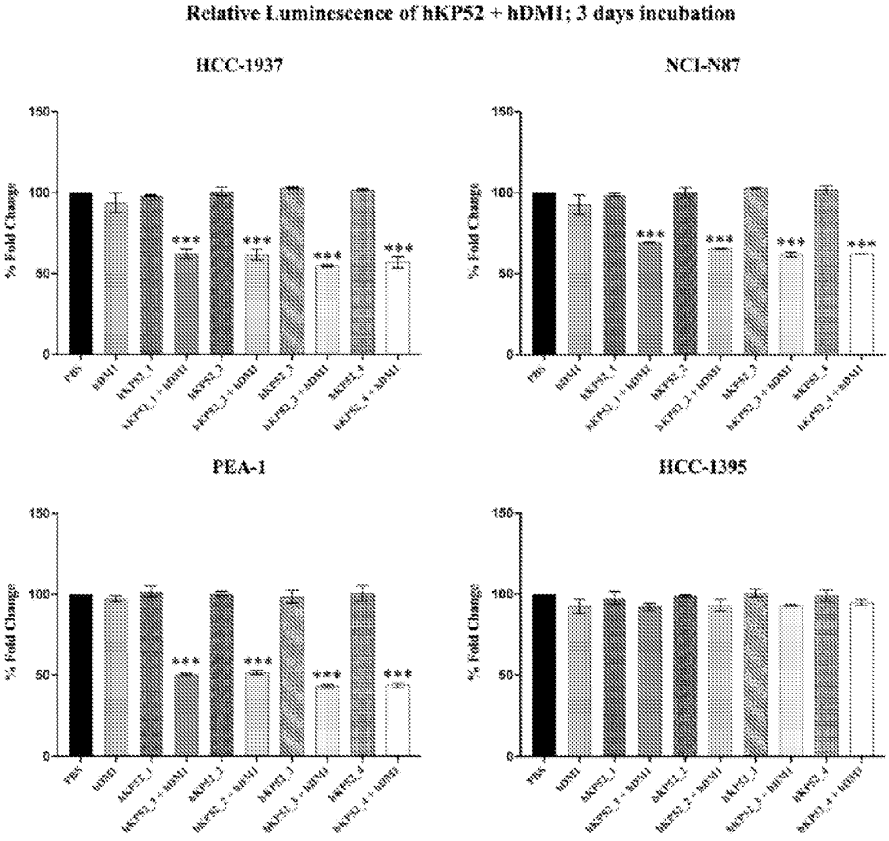

Relative Luminescence of hKP52 + hDM1; 3 days incubation
HCC-1937
NCI-N87
PEA-1
HCC-1395
% Fold Change
150
100
50
0
***

Figure 19

mAbs	k_{on} (1/Ms)	k_{off} (1/s)	K_D (nM)
KP52	8.97×10^{5}	1.49×10^{-4}	0.166
hKP52_1	1.02×10^{6}	1.96×10^{-3}	1.921
hKP52_2	1.16×10^{6}	2.39×10^{-3}	2.062
hKP52_3	9.80×10^{5}	7.20×10^{-4}	0.734
hKP52_4	9.71×10^{5}	9.75×10^{-4}	1.004

Figure 20

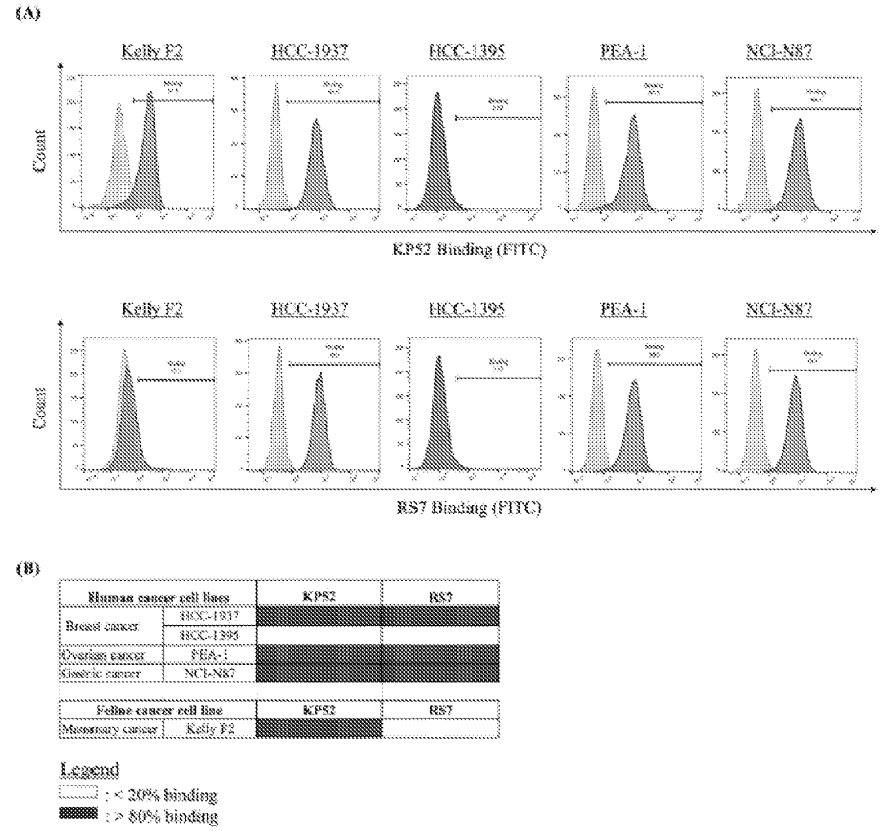

Comparing KP52 with RS7
(A)
Kelly F2
HCC-1937
HCC-1395
PEA-1
NCI-N87
Count
KP52 Binding (FITC)
RS7 Binding (FITC)
(B)
Human cancer cell lines
KP52
RS7
Breast cancer
HCC-1937
HCC-1395
Ovarian cancer
PEA-1
Gastric cancer
NCI-N87
Feline cancer cell line
Mammary cancer
Kelly F2
Legend
: < 20% binding
: > 80% binding

Figure 21

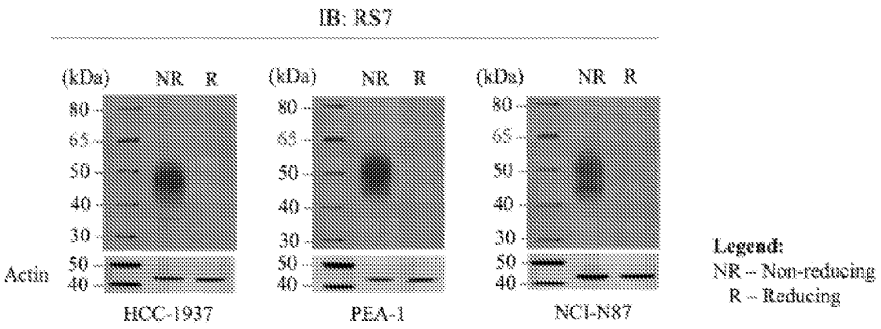

IB: RS7
(kDa)
NR
R
80
65
50
40
30
50
40
Actin
HCC-1937
PEA-1
NCI-N87
Legend:
NR – Non-reducing
R – Reducing

Figure 22

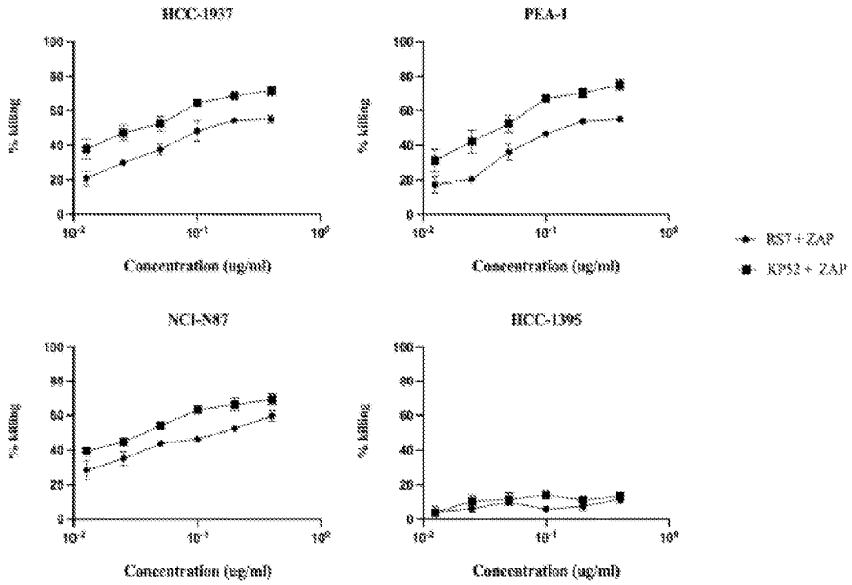

HCC-1937
PEA-1
NCI-N87
HCC-1395
% killing
Concentration (ug/ml)
BS7 + ZAP
KP52 + ZAP

Figure 23

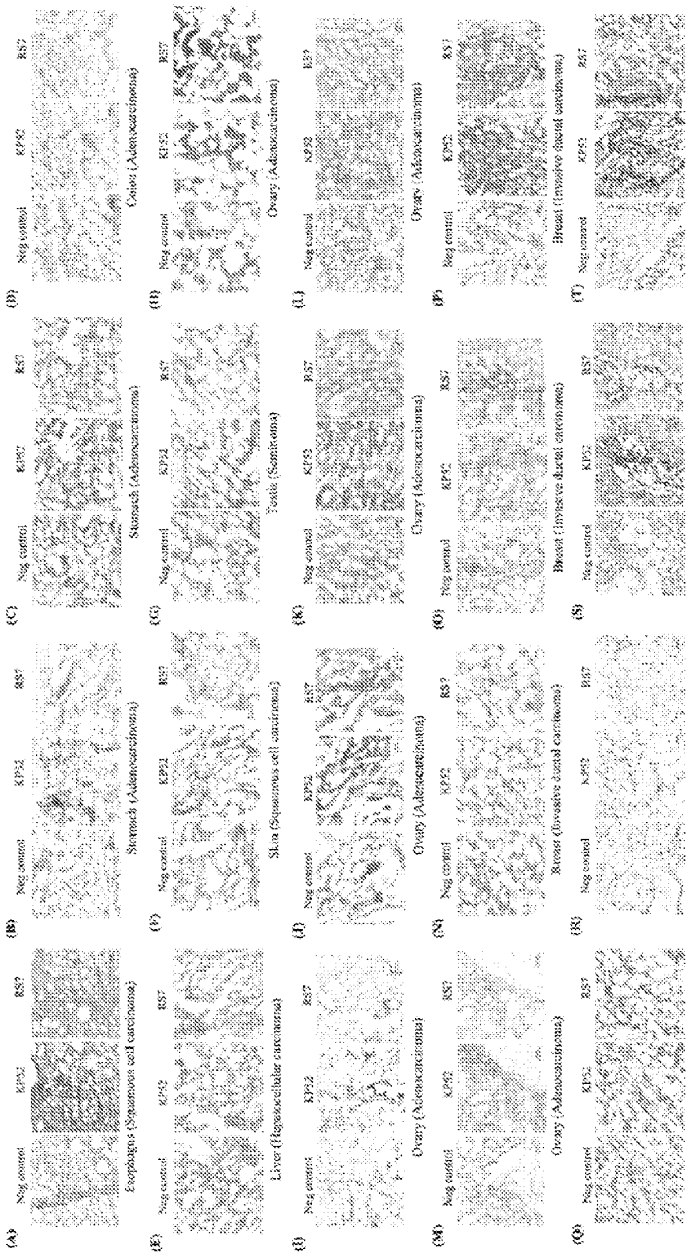

(A)
Neg control
KP52
RS7
Esophagus (Squamous cell carcinoma)
(B)
Neg control
KP52
RS7
Stomach (Adenocarcinoma)
(C)
Neg control
KP52
RS7
Stomach (Adenocarcinoma)
(D)
Neg control
KP52
RS7
Cervix (Adenocarcinoma)
(E)
Neg control
KP52
RS7
Liver (Hepatocellular carcinoma)
(F)
Neg control
KP52
RS7
Skin (Squamous cell carcinoma)
(G)
Neg control
KP52
RS7
Testis (Seminoma)
(H)
Neg control
KP52
RS7
Ovary (Adenocarcinoma)
(I)
Neg control
KP52
RS7
Ovary (Adenocarcinoma)
(J)
Neg control
KP52
RS7
Ovary (Adenocarcinoma)
(K)
Neg control
KP52
RS7
Ovary (Adenocarcinoma)
(L)
Neg control
KP52
RS7
Ovary (Adenocarcinoma)
(M)
Neg control
KP52
RS7
Ovary (Adenocarcinoma)
(N)
Neg control
KP52
RS7
Breast (Invasive ductal carcinoma)
(O)
Neg control
KP52
RS7
Breast (Invasive ductal carcinoma)
(P)
Neg control
KP52
RS7
Breast (Invasive ductal carcinoma)
(Q)
Neg control
KP52
RS7
Oral cavity (Ameloblastoma of hyperplastic)
(R)
Neg control
KP52
RS7
Kidney (Clear cell carcinoma)
(S)
Neg control
KP52
RS7
Lung (Adenocarcinoma)
(T)
Neg control
KP52
RS7
Lung (Squamous cell carcinoma)

Figure 24

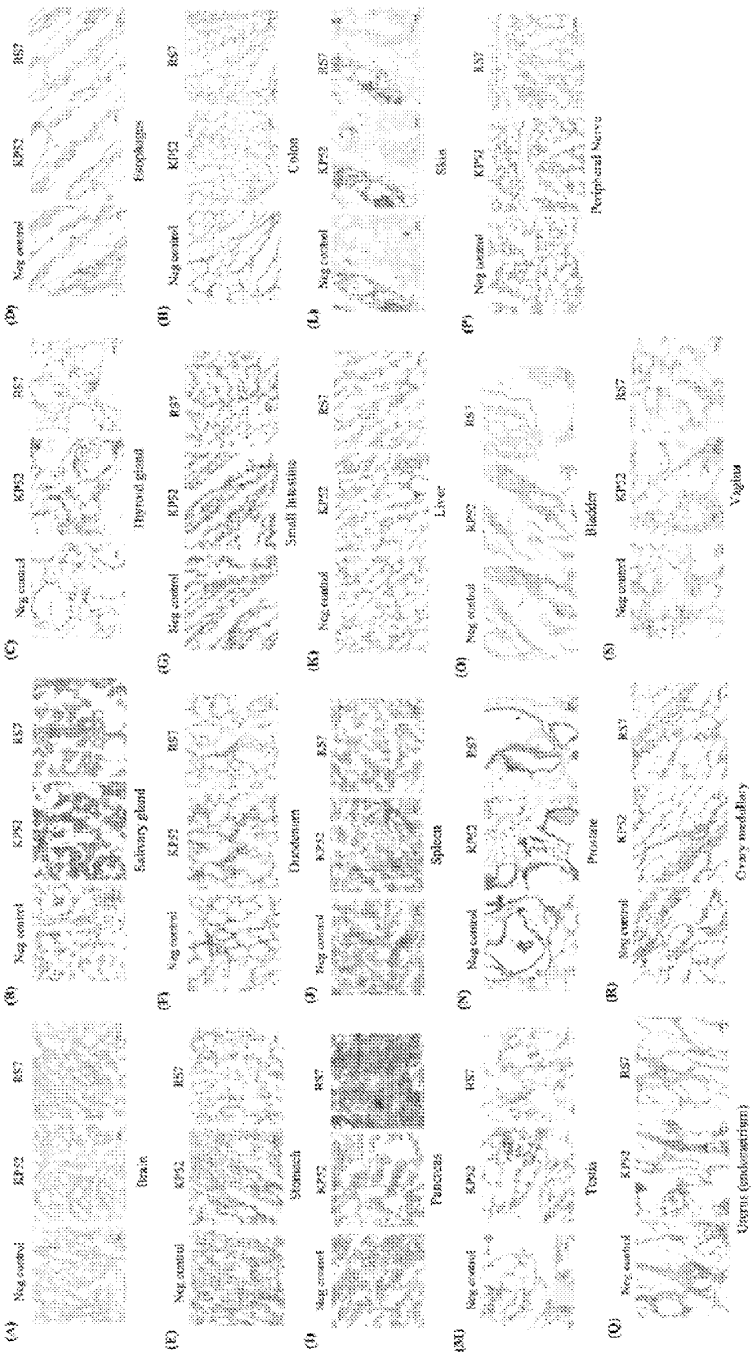

(A)
Neg control
KP52
RS7
Brain
(B)
Neg control
KP52
RS7
Salivary gland
(C)
Neg control
KP52
RS7
Thyroid gland
(D)
Neg control
KP52
RS7
Esophagus
(E)
Neg control
KP52
RS7
Stomach
(F)
Neg control
KP52
RS7
Duodenum
(G)
Neg control
KP52
RS7
Small Intestine
(H)
Neg control
KP52
RS7
Colon
(I)
Neg control
KP52
RS7
Pancreas
(J)
Neg control
KP52
RS7
Spleen
(K)
Neg control
KP52
RS7
Liver
(L)
Neg control
KP52
RS7
Skin
(M)
Neg control
KP52
RS7
Testis
(N)
Neg control
KP52
RS7
Prostate
(O)
Neg control
KP52
RS7
Bladder
(P)
Neg control
KP52
RS7
Peripheral Nerve
(Q)
Neg control
KP52
RS7
Uterus (endometrium)
(R)
Neg control
KP52
RS7
Ovary medullary
(S)
Neg control
KP52
RS7
Vagina

Figure 25

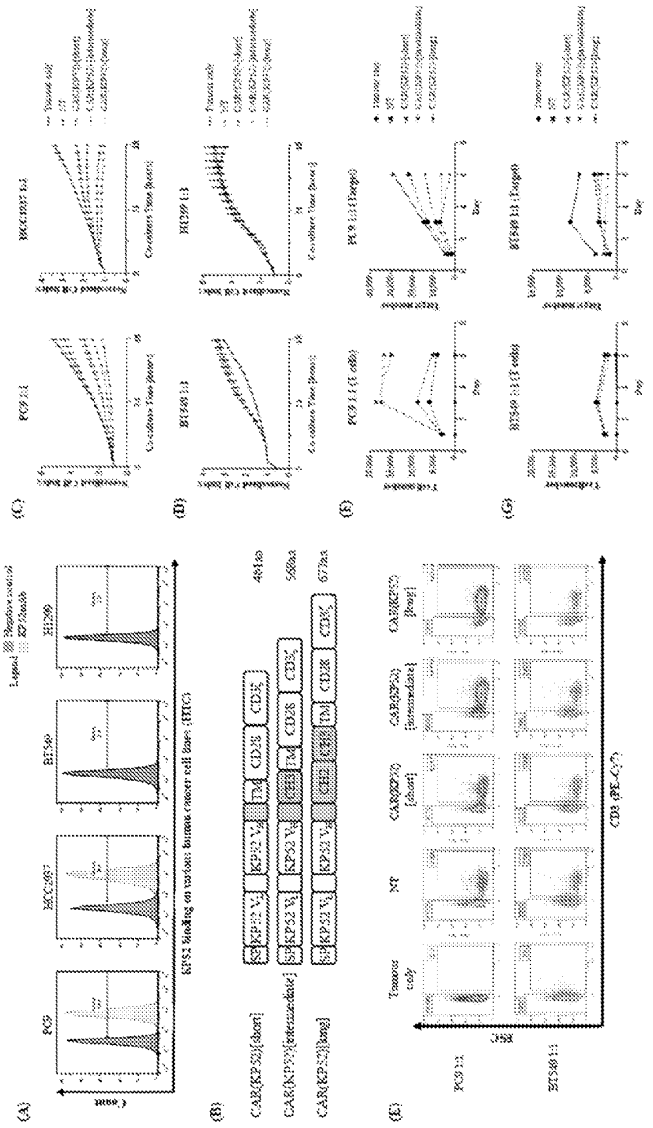


Figure 26

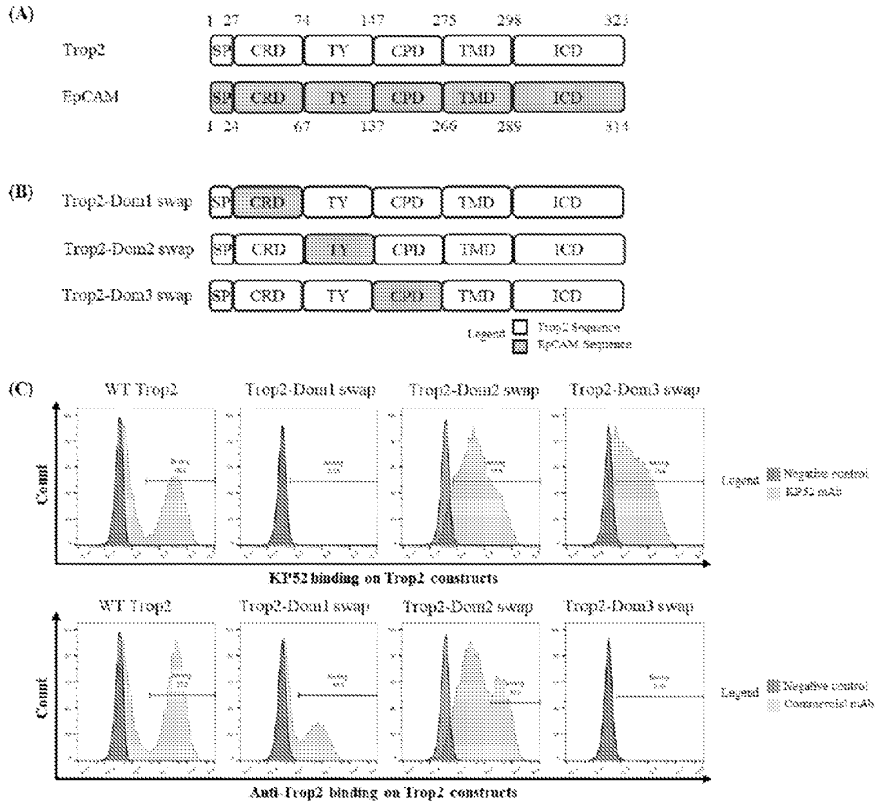

(A)
Trop2
EpCAM
SP
CRD
TY
CPD
TMD
ICD
(B)
Trop2-Dom1 swap
Trop2-Dom2 swap
Trop2-Dom3 swap
Legend
Trop2 Sequence
EpCAM Sequence
(C)
WT Trop2
Trop2-Dom1 swap
Trop2-Dom2 swap
Trop2-Dom3 swap
Count
Legend
Negative control
KP52 mAb
KP52 binding on Trop2 constructs
Commercial mAb
Anti-Trop2 binding on Trop2 constructs

Figure 27

Trop2 Protein Sequences

>Human TROP2-CRD

HTAAQDNCTCPTNKMTVCSPDGPGGRCQCRALGSGMAVDCSTLTSKC (SEQ ID NO: 26)

>Feline TROP2-CRD

PAAAQDNCTCATNKMTLCRADGPGGRCQCRALGSGFPVDCSTLTSKC (SEQ ID NO 27)

>tr|M3VZE2|M3VZE2_FELCA Tumor associated calcium signal transducer 2 OS=Felis catus OX=9685 GN=TACSTD2 PE=3 SV=3

MAREPGLALPSLPLPLLLLLLLAAATGPAAAQDNCTCATNKMTLCRADGPGGRCQCRALGSGFPVD
CSTLTSKCLLLKARMSTPKSGRALVRPSEHALVDNDGLYDPDCDHEGRFKARQCNQTSVCWCVNSV
GVRRTDKGDLSLRCDELVRTHHILIDLRHRPAARAFNHSDLDAELRRLFRERYRLHPRFVAAVHYE
RPTIQIELRQNASEKAPGDVDIADAAYYFERDVKGESLFPGRGGLDVRVRGEPLLVERTLIYYLDE
KPPEFSMKRLTAGLIAVIVVVVVALVAGVAVLVITNRRKSGKYKKVEVKELGELRKEPSL (SEQ
ID NO: 28)

Figure 28