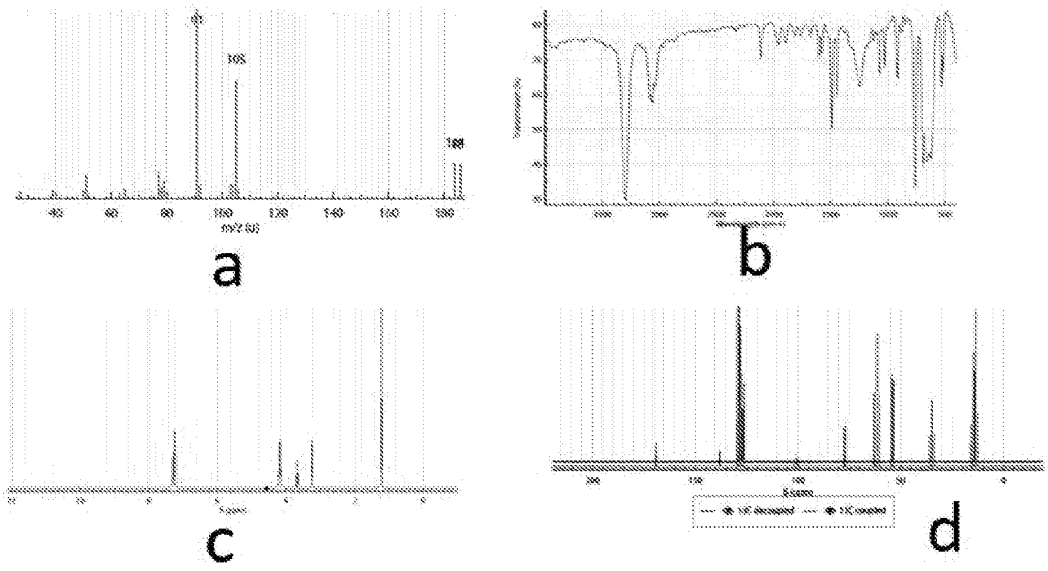


Figure 1

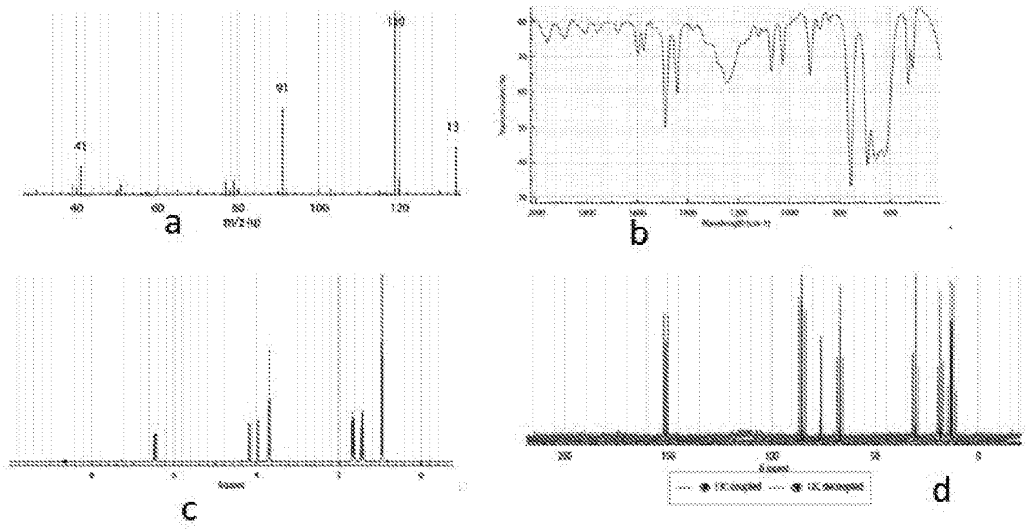


Figure 2

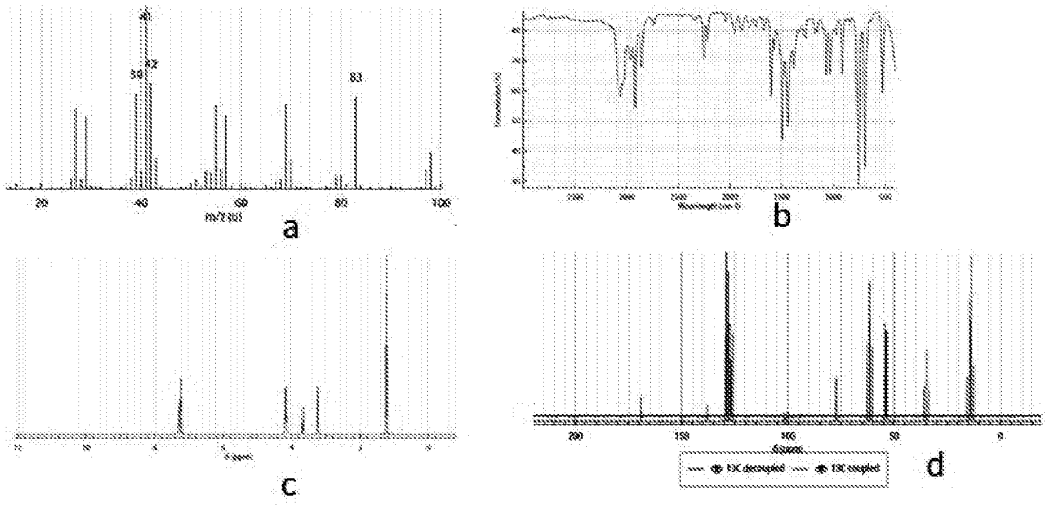


Figure 3

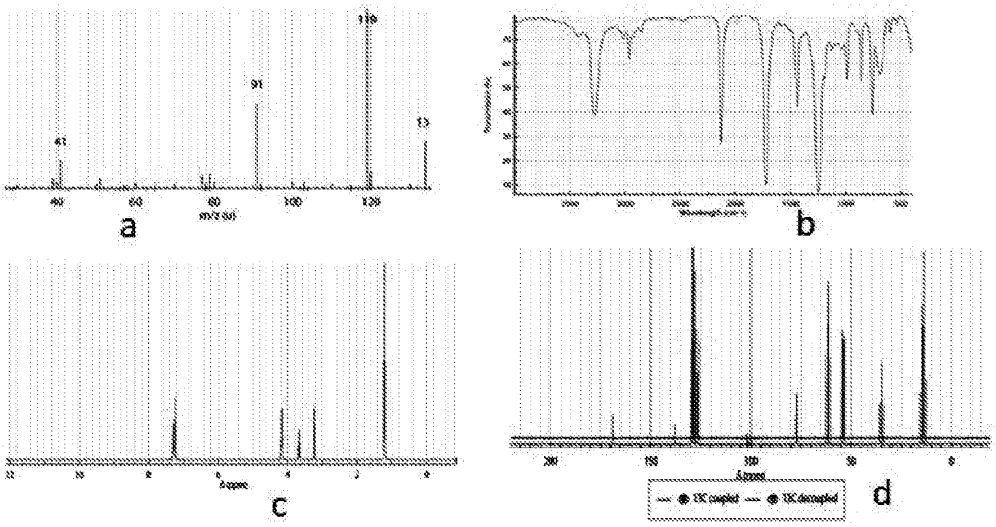


Figure 4

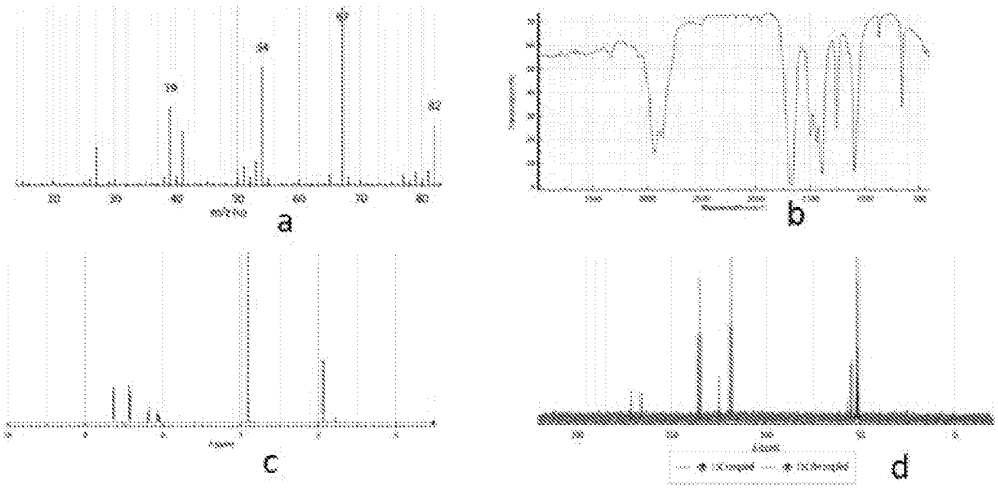


Figure 5

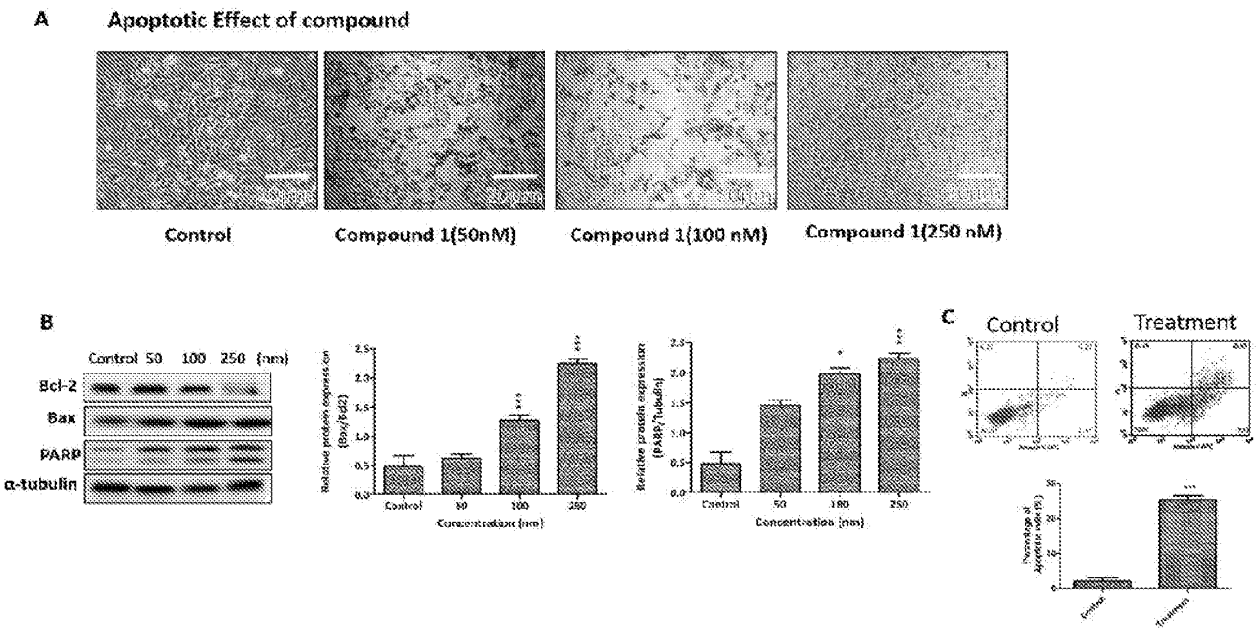
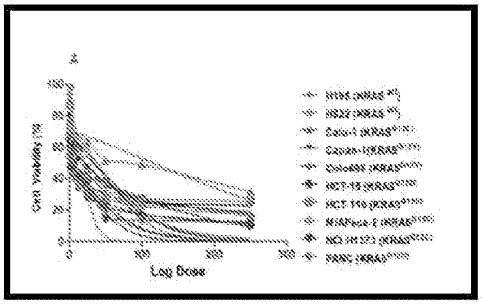

A
Apoptotic Effect of compound
Control
Compound 1(50nM)
Compound 1(100 nM)
Compound 1(250 nM)
B
Control 50 100 250 (nm)
Bcl-2
Bax
PARP
α-tubulin
C
Control
Treatment

Figure 6

A



B

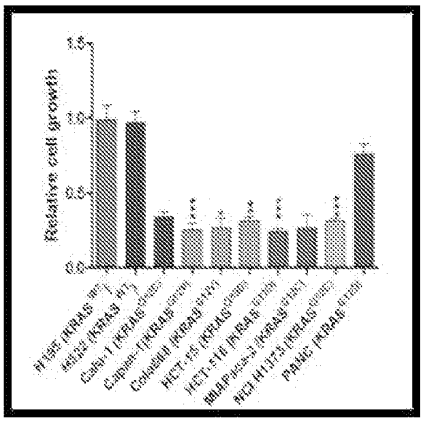


Figure 7

S

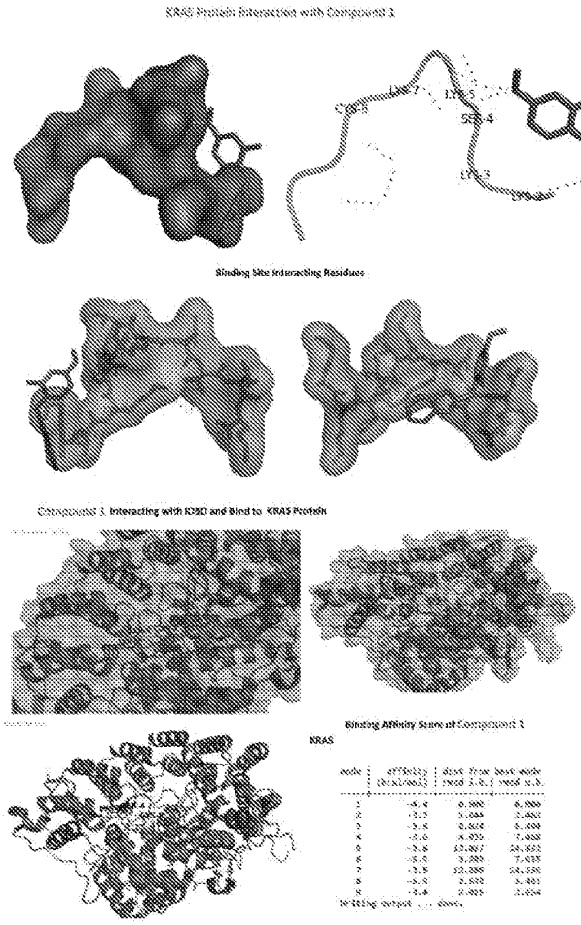

KRAS Protein Interaction with Component 3
Binding Site Interacting Residues
Component 3 Interacting with R38D and Bind to KRAS Protein
Binding Affinity Score of Component 3
KRAS

Figure 8

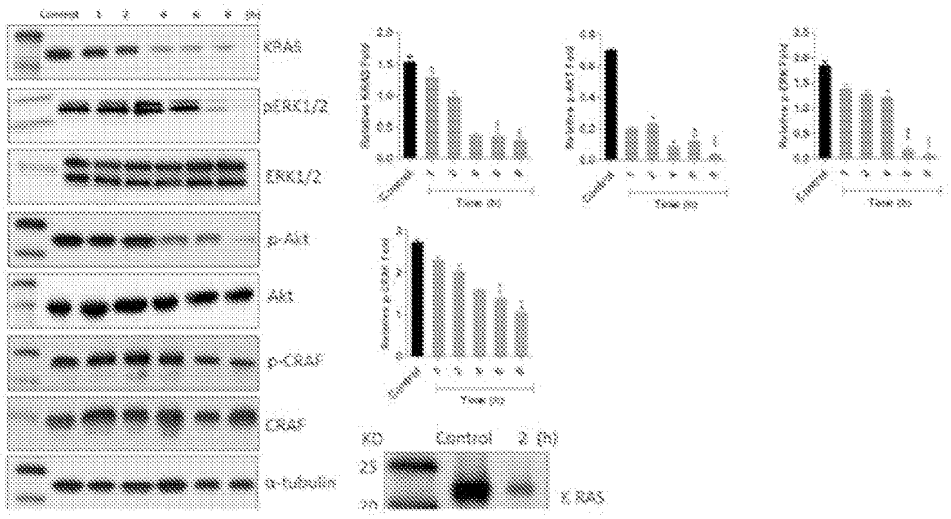
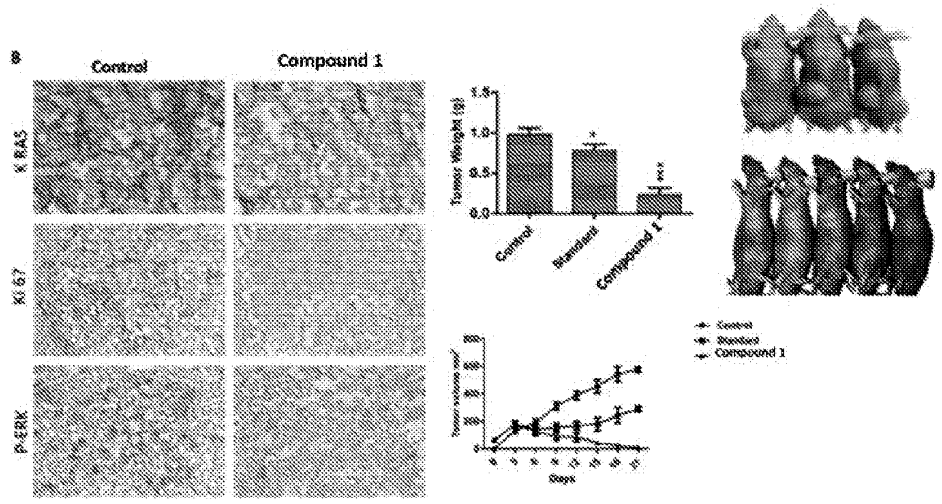
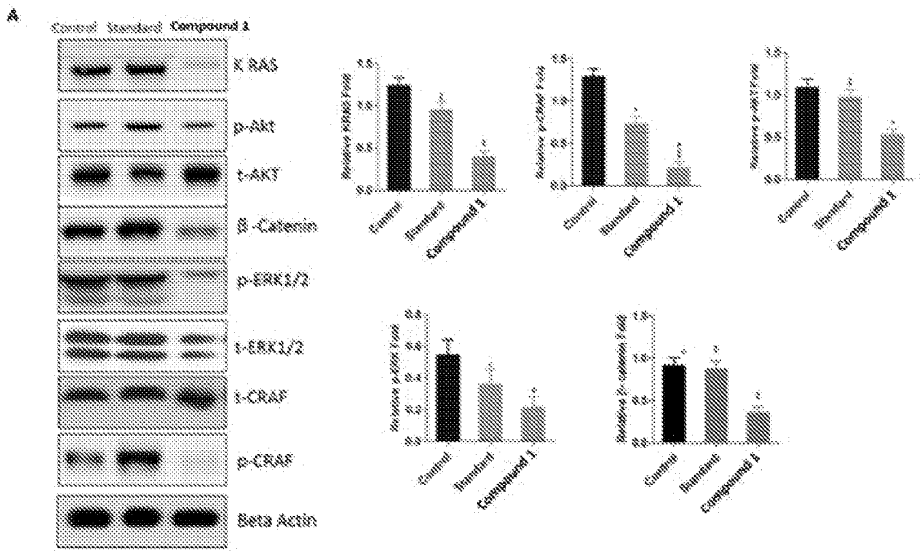

Control
KRAS
pERK1/2
ERK1/2
p-AKT
AKT
p-CRAF
CRAF
α-tubulin
Time (h)
KO
Control
2 (h)
K RAS

Figure 9


B
Control
Compound 1
K RAS
Ki 67
p-ERK
Control
Standard
Compound 1

a


A
Control
Standard
Compound 1
K RAS
p-Akt
t-AKT
β -Catenin
p-ERK1/2
t-ERK1/2
t-CRAF
p-CRAF
Beta Actin

b

Figure 10

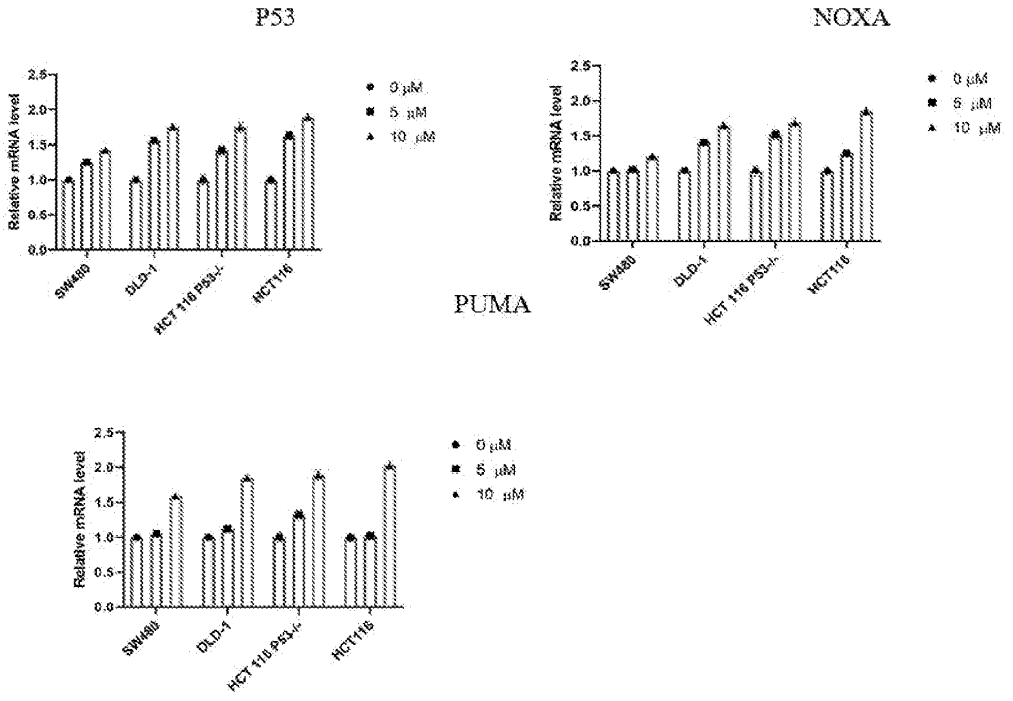

P53
NOXA
PUMA
Relative mRNA level
0 μM
5 μM
10 μM
SW480
DLD-1
HCT 116 P53-/-
HCT116

Figure 11

s

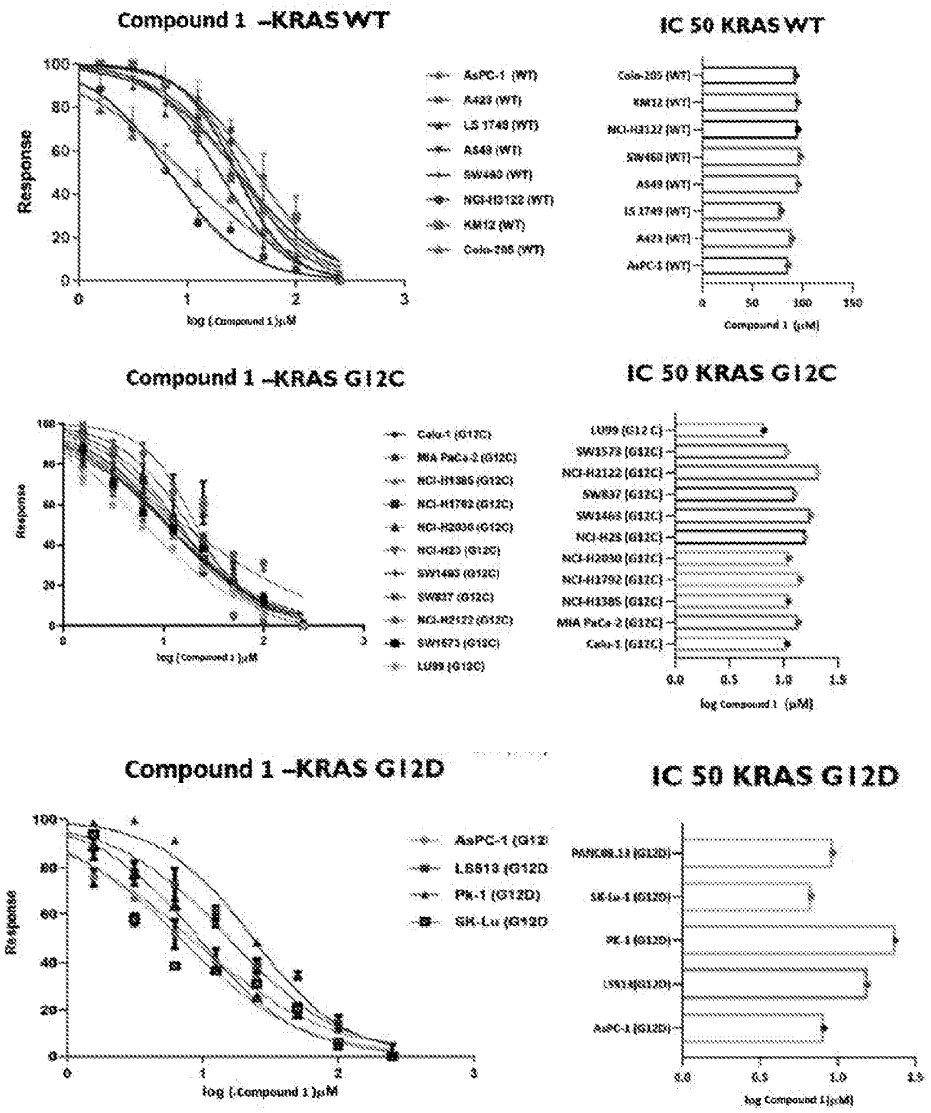

Compound 1 --KRAS WT
IC 50 KRAS WT
Response
AsPC-1 (WT)
A423 (WT)
LS 1748 (WT)
A549 (WT)
SW480 (WT)
NCI-H3122 (WT)
KM12 (WT)
Colo-205 (WT)
Compound 1 --KRAS G12C
IC 50 KRAS G12C
Colo-1 (G12C)
MIA PaCa-2 (G12C)
NCI-H1385 (G12C)
NCI-H1792 (G12C)
NCI-H2030 (G12C)
NCI-H23 (G12C)
SW1463 (G12C)
SW837 (G12C)
NCI-H2122 (G12C)
SW1573 (G12C)
LU99 (G12C)
Compound 1 --KRAS G12D
IC 50 KRAS G12D
AsPC-1 (G12D)
LS513 (G12D)
PK-1 (G12D)
SK-Lu (G12D)

Figure 12

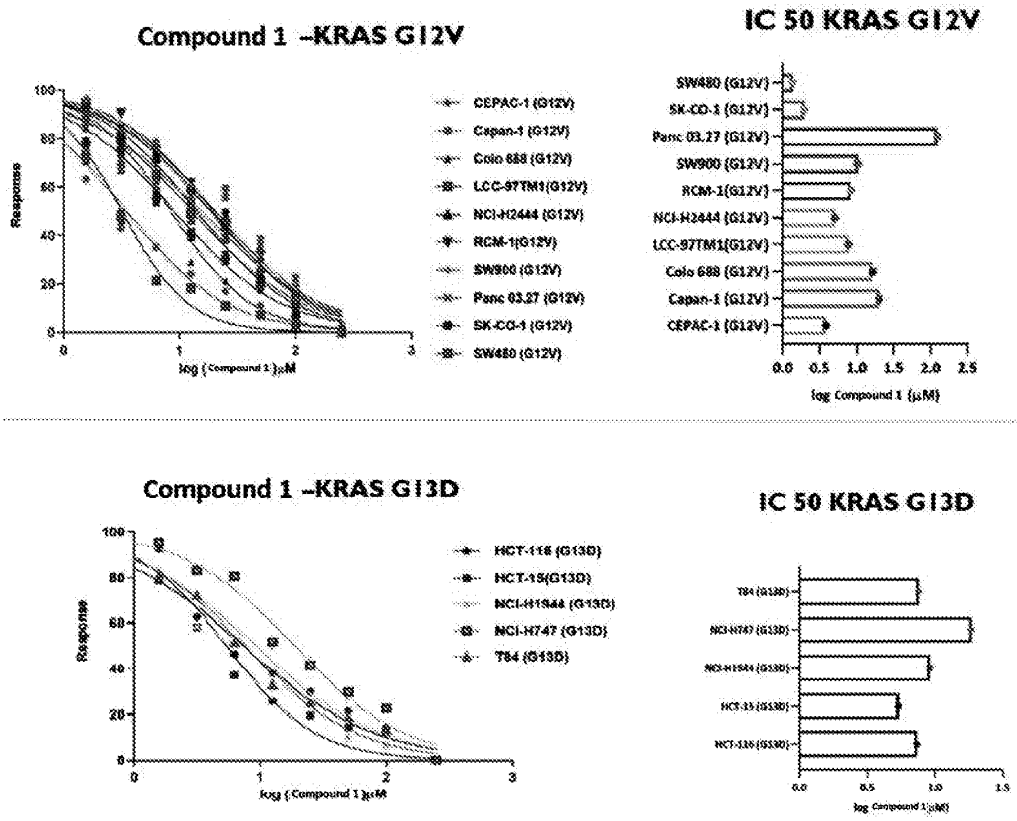


Figure 12 (Continued)

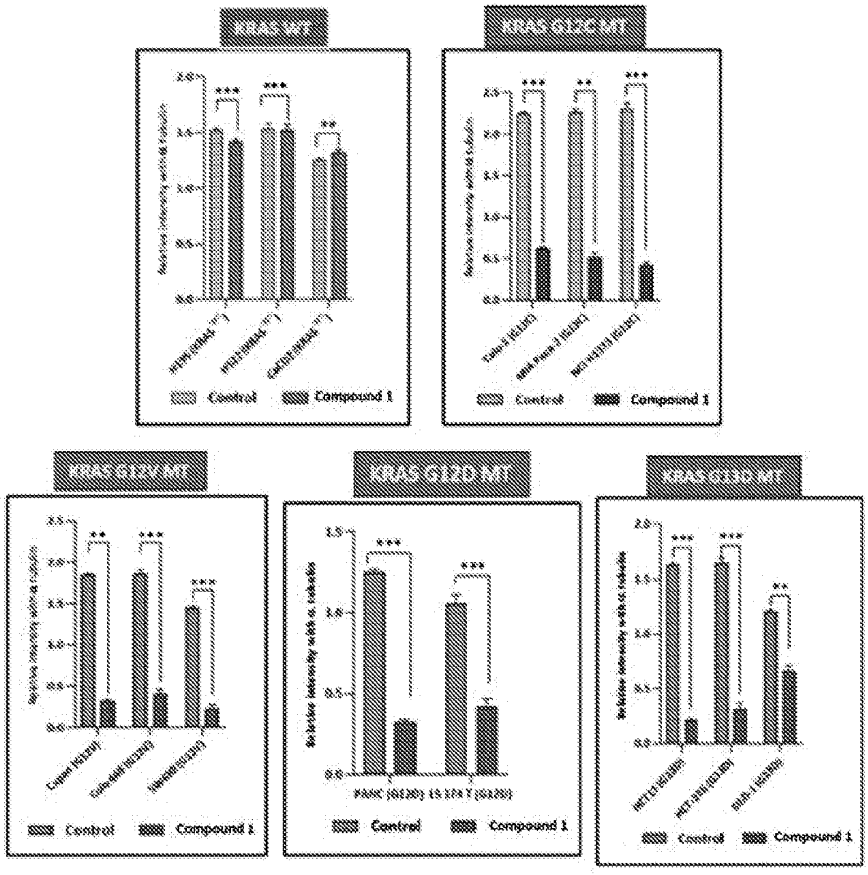

KRAS MT
KRAS G12C MT
KRAS G12V MT
KRAS G12D MT
KRAS G13D MT
Control
Compound 1

Figure 13

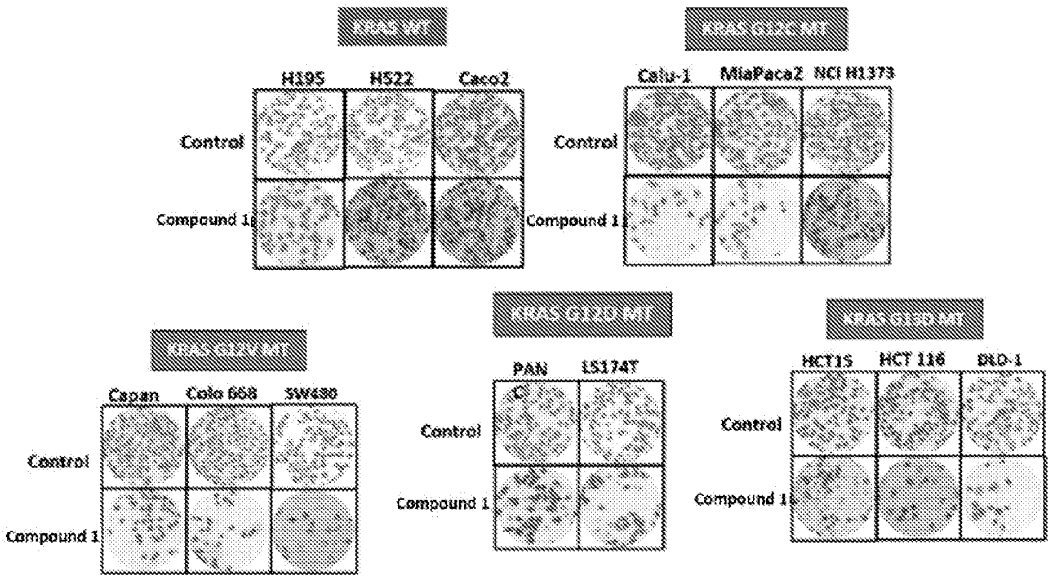


Figure 13 (Continued)

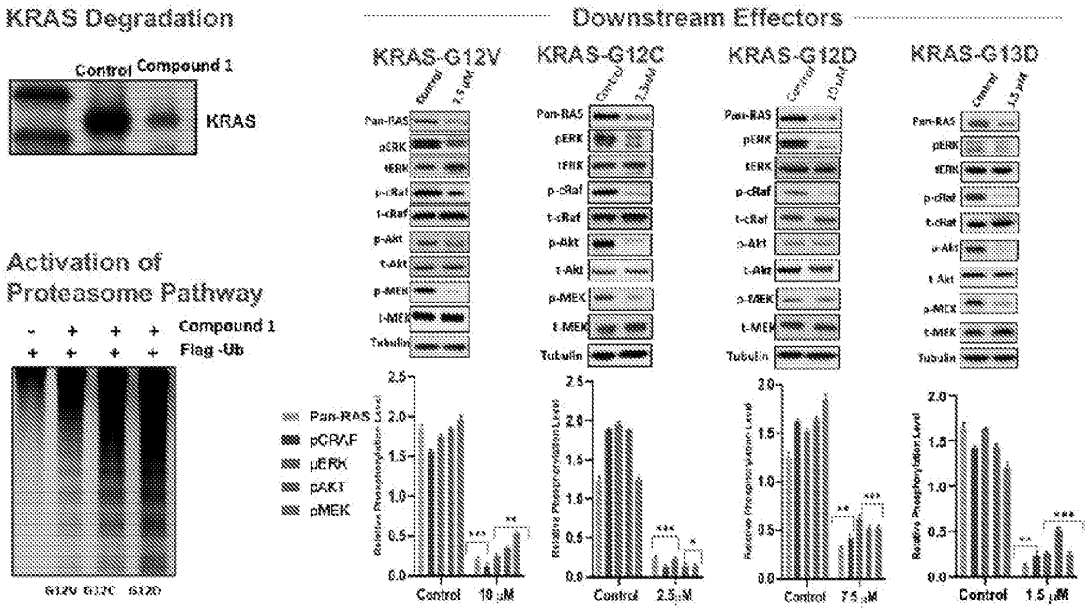

KRAS Degradation
Control
Compound 1
KRAS
Activation of Proteasome Pathway
Compound 1
Flag-Ub
Downstream Effectors
KRAS-G12V
KRAS-G12C
KRAS-G12D
KRAS-G13D
Pan-RAS
pERK
tERK
p-cRaf
t-cRaf
p-Akt
t-Akt
p-MEK
t-MEK
Tubulin
pCRAF
pAKT
pMEK
Relative Phosphorylation Level
Control
10 μM
2.5 μM
7.5 μM
1.5 μM

Figure 14

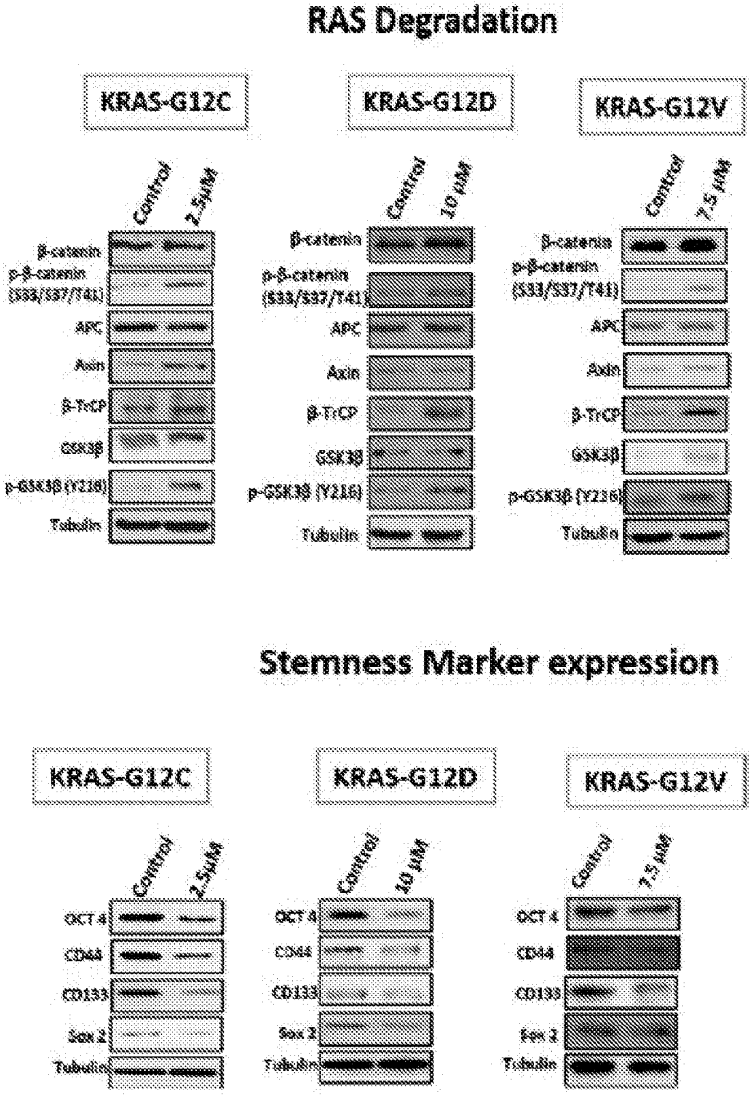

RAS Degradation
KRAS-G12C
KRAS-G12D
KRAS-G12V
Control
2.5μM
10 μM
7.5 μM
β-catenin
p-β-catenin (S33/S37/T41)
APC
Axin
β-TrCP
GSK3β
p-GSK3β (Y216)
Tubulin
Stemness Marker expression
KRAS-G12C
KRAS-G12D
KRAS-G12V
Control
2.5μM
10 μM
7.5 μM
OCT 4
CD44
CD133
Sox 2
Tubulin

Figure 15

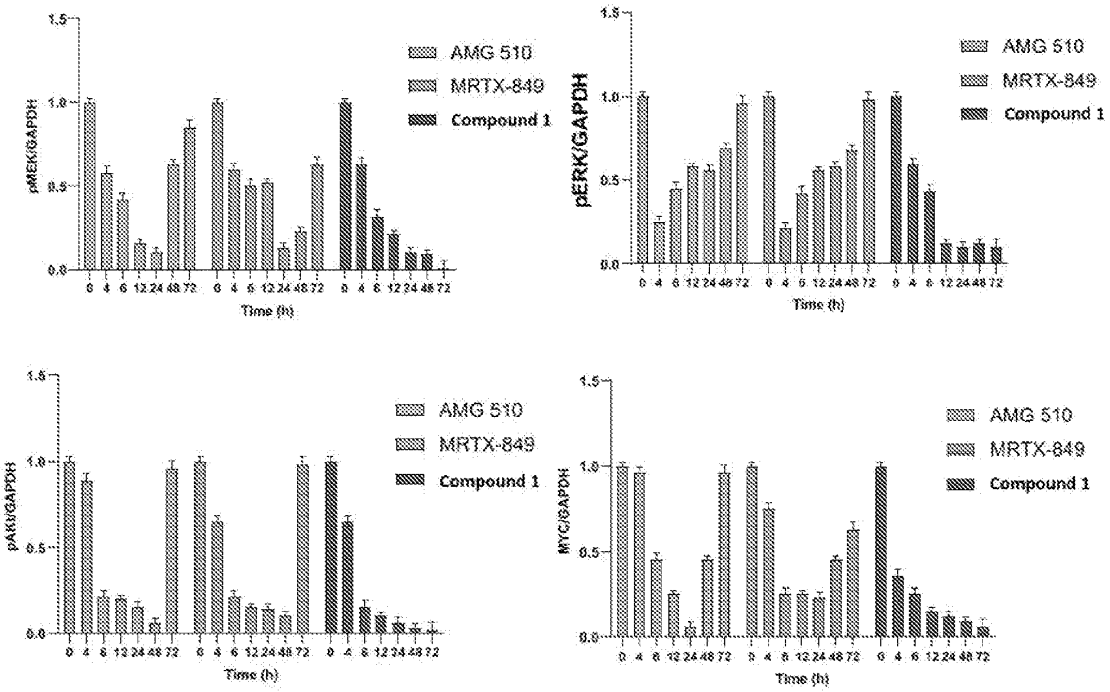

pMEK/GAPDH
pERK/GAPDH
pAKt/GAPDH
MYC/GAPDH
AMG 510
MRTX-849
Compound 1
Time (h)

Figure 16

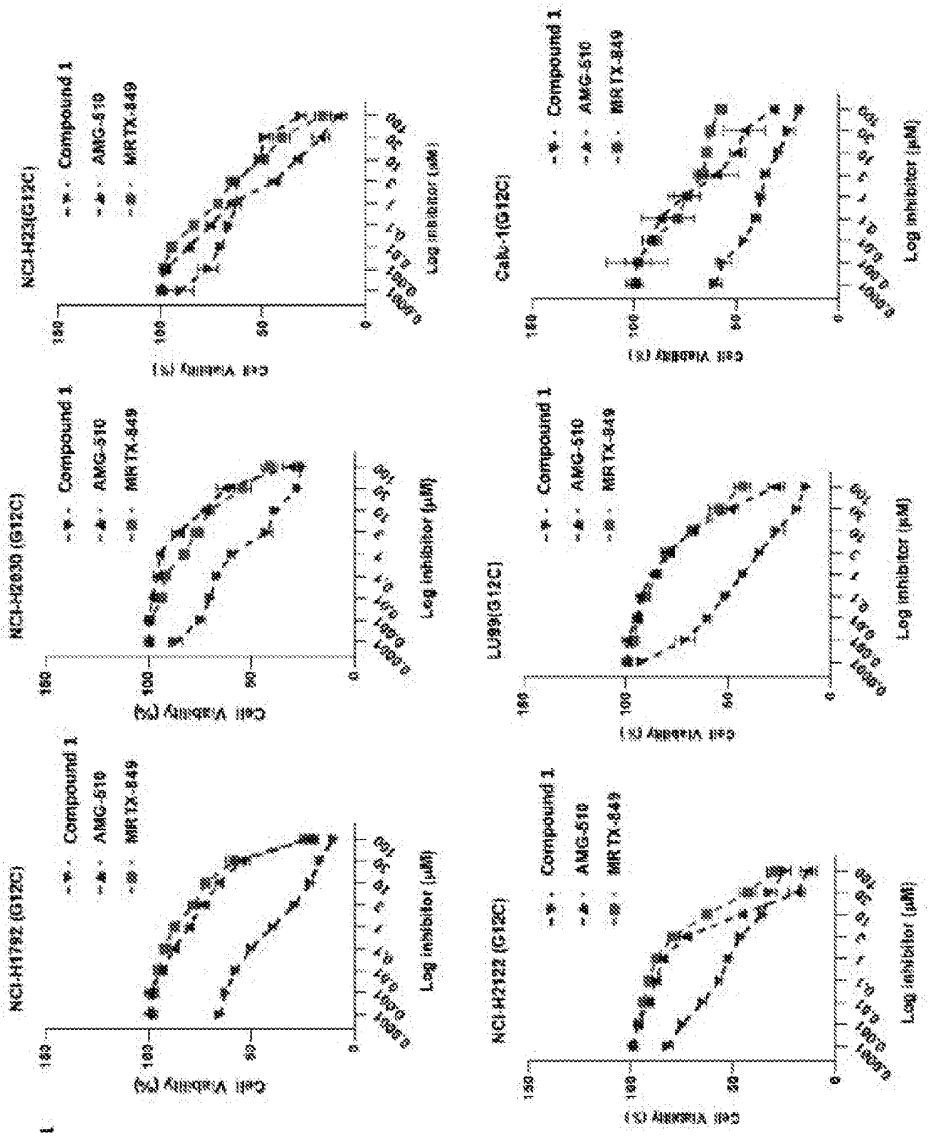

NCI-H1792 (G12C)
NCI-H2030 (G12C)
NCI-H23(G12C)
LU99(G12C)
Calu-1(G12C)
NCI-H2122 (G12C)
Compound 1
AMG-510
MRTX-849
Cell Viability (%)
Log inhibitor (μM)

Figure 17

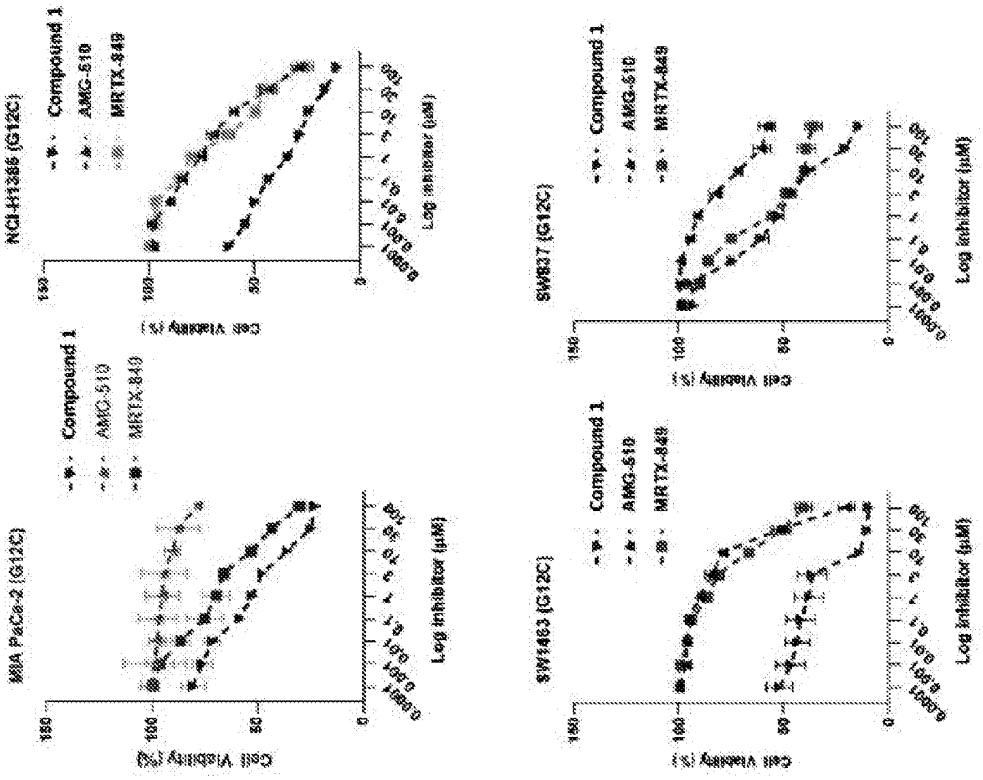


Figure 17 (Continued)

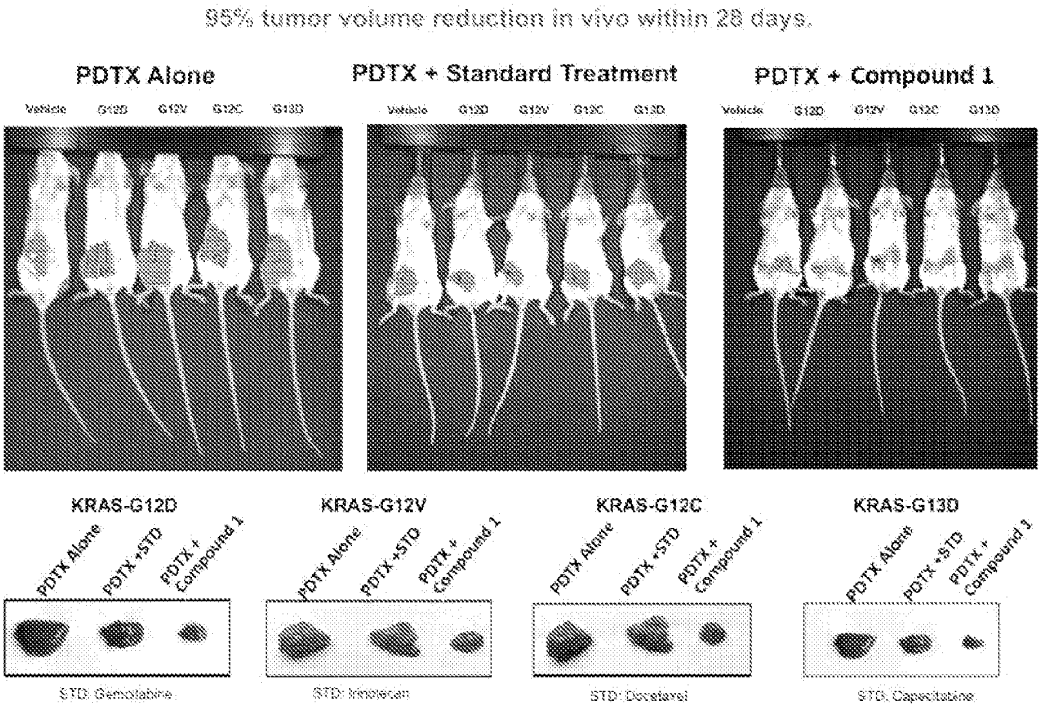

95% tumor volume reduction in vivo within 28 days.
PDTX Alone
PDTX + Standard Treatment
PDTX + Compound 1
Vehicle
G12D
G12V
G12C
G13D
KRAS-G12D
KRAS-G12V
KRAS-G12C
KRAS-G13D
PDTX Alone
PDTX +STD
PDTX + Compound 1
STD: Gemcitabine
STD: Irinotecan
STD: Docetaxel
STD: Capecitabine

Figure 18

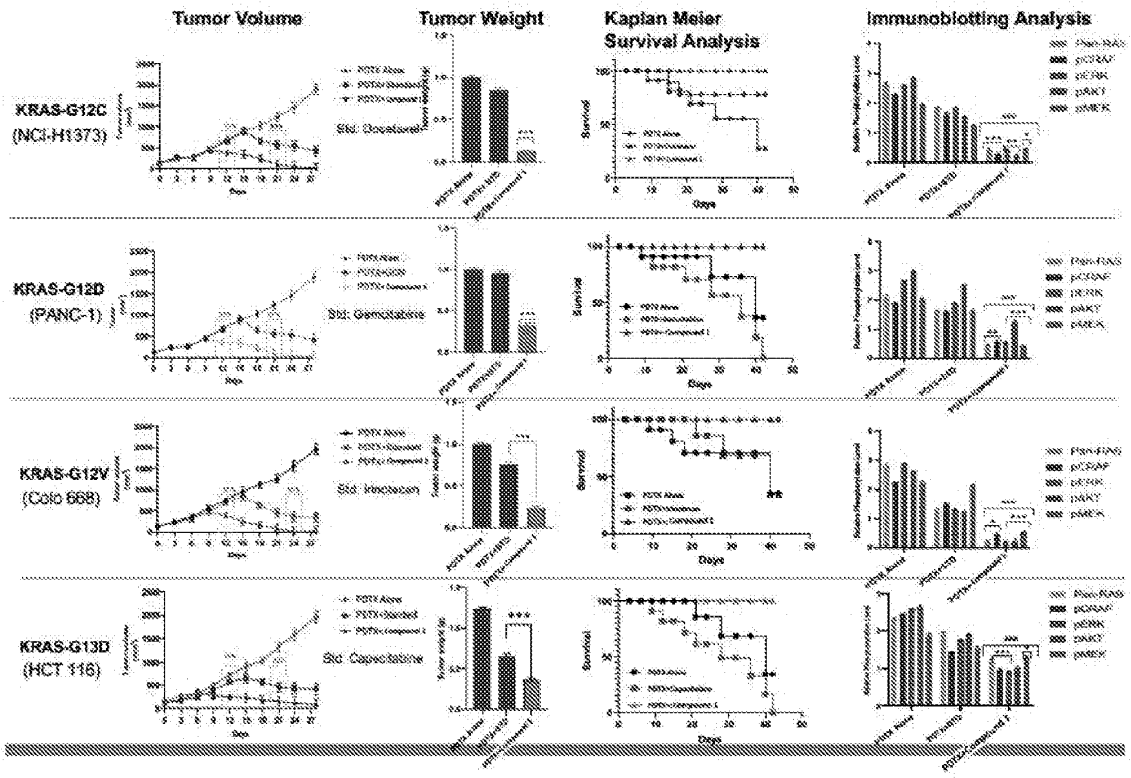

Tumor Volume
Tumor Weight
Kapian Meier Survival Analysis
Immunoblotting Analysis
KRAS-G12C (NCI-H1373)
KRAS-G12D (PANC-1)
KRAS-G12V (Colo 668)
KRAS-G13D (HCT 116)
Std. Docetaxel
Std. Gemcitabine
Std. Irinotecan
Std. Capecitabine
Days
Survival
Phos-RAS
pCRAF
pERK
pAKT
pMEK

Figure 19

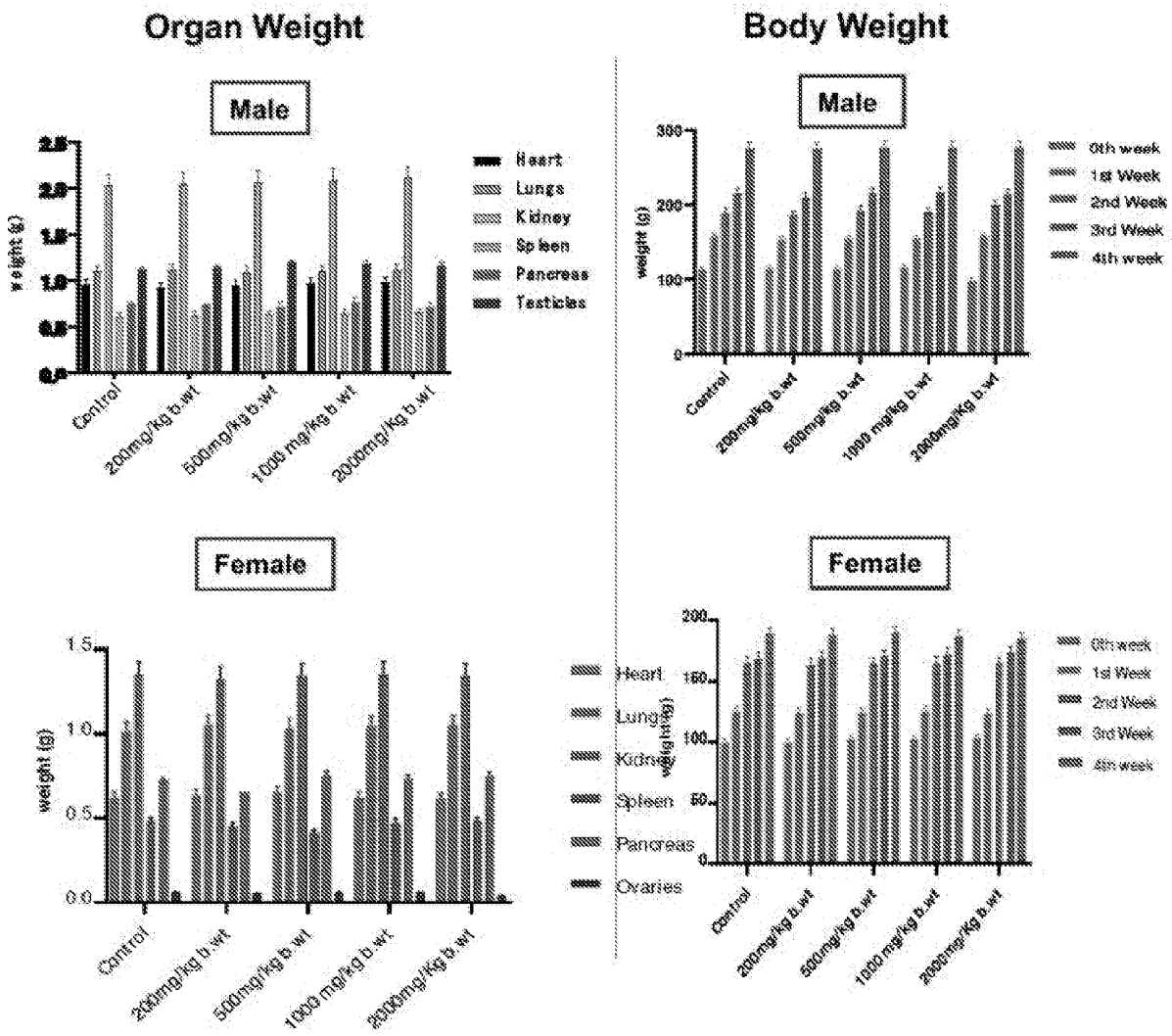

Organ Weight
Male
Heart
Lungs
Kidney
Spleen
Pancreas
Testicles
Control
200mg/kg b.wt
500mg/kg b.wt
1000 mg/kg b.wt
2000mg/kg b.wt
Female
weight (g)
Body Weight
Male
0th week
1st Week
2nd Week
3rd Week
4th week
Female
Ovaries

Figure 20

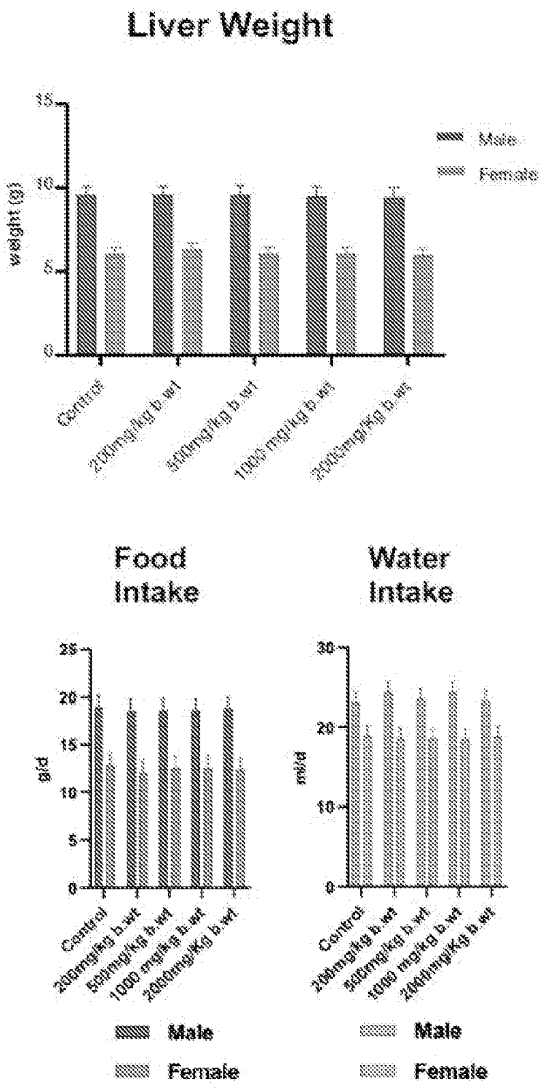


Figure 20 (Continued)

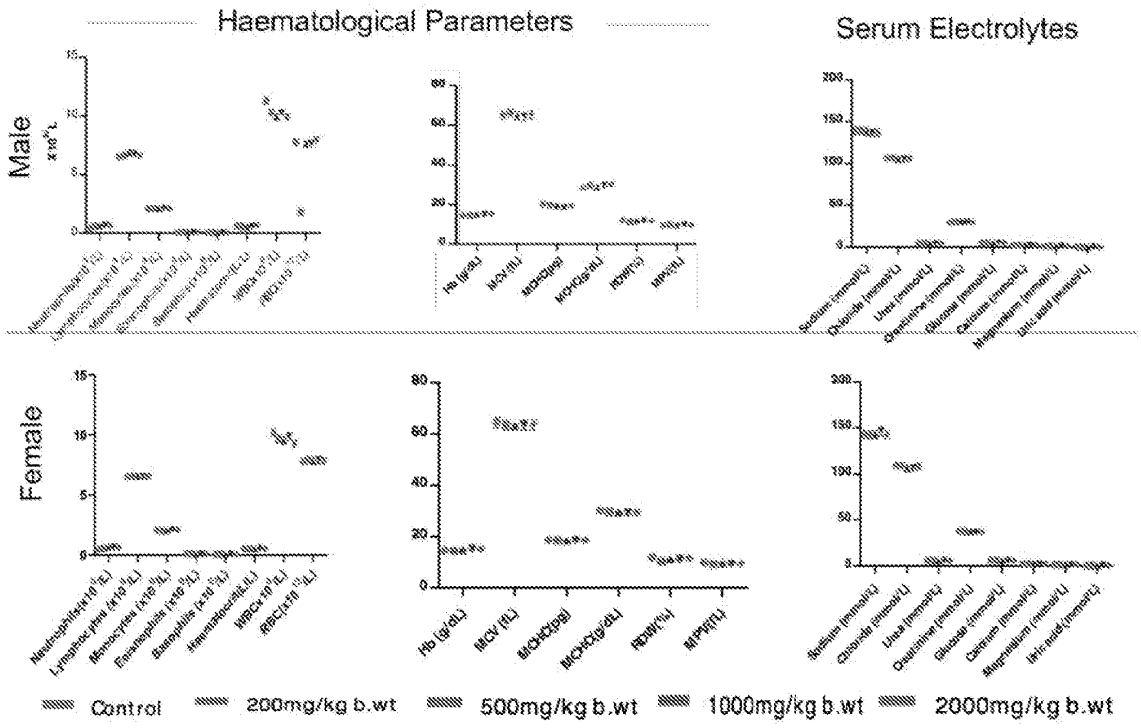


Figure 20 (Continued)

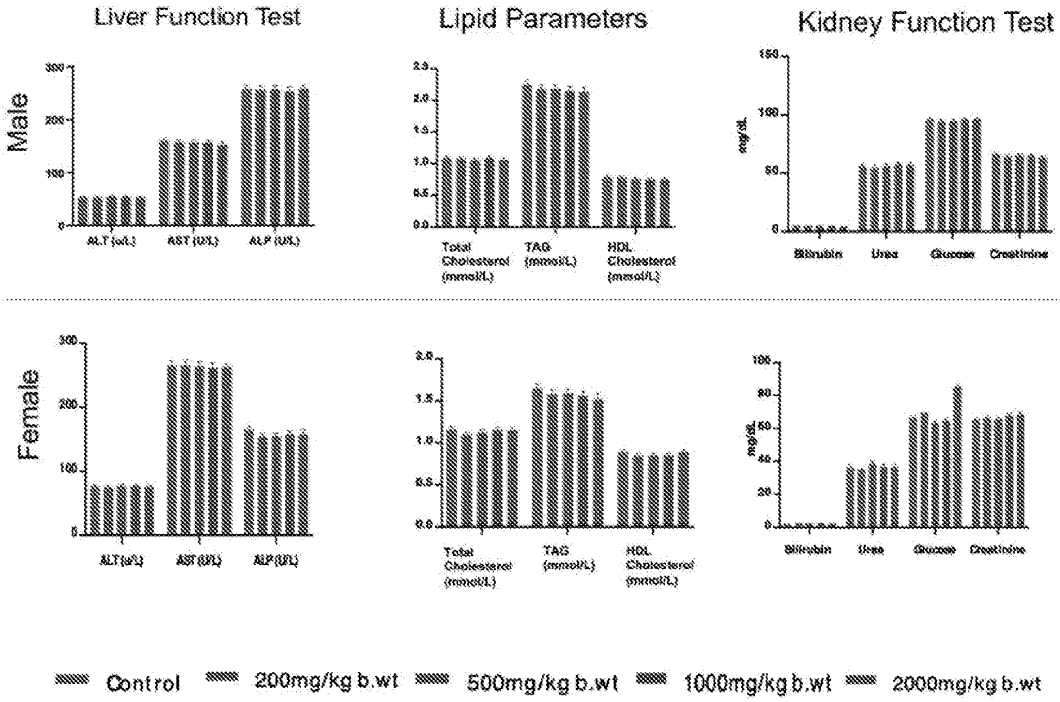


Figure 20 (Continued)

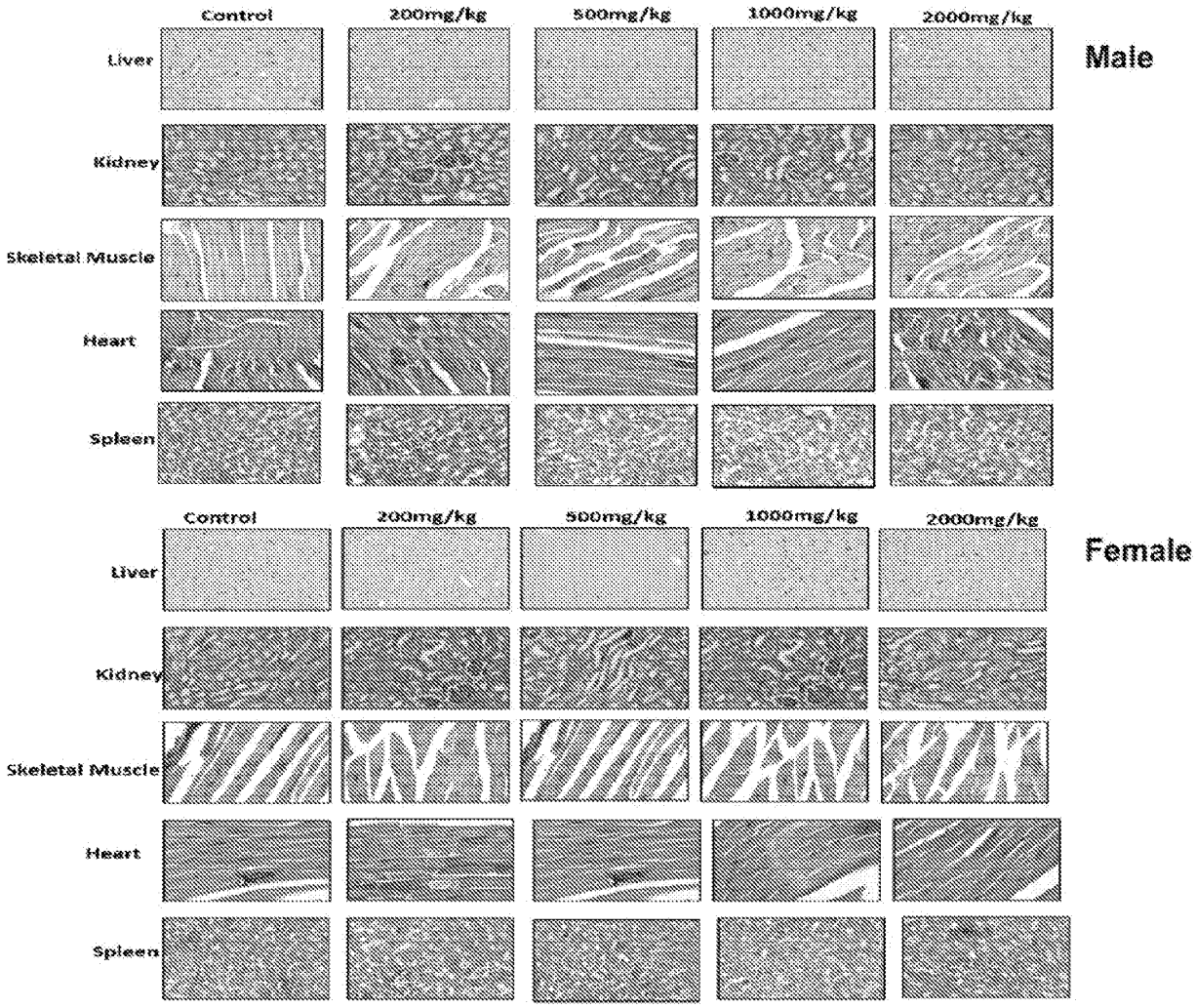


Figure 20 (Continued)