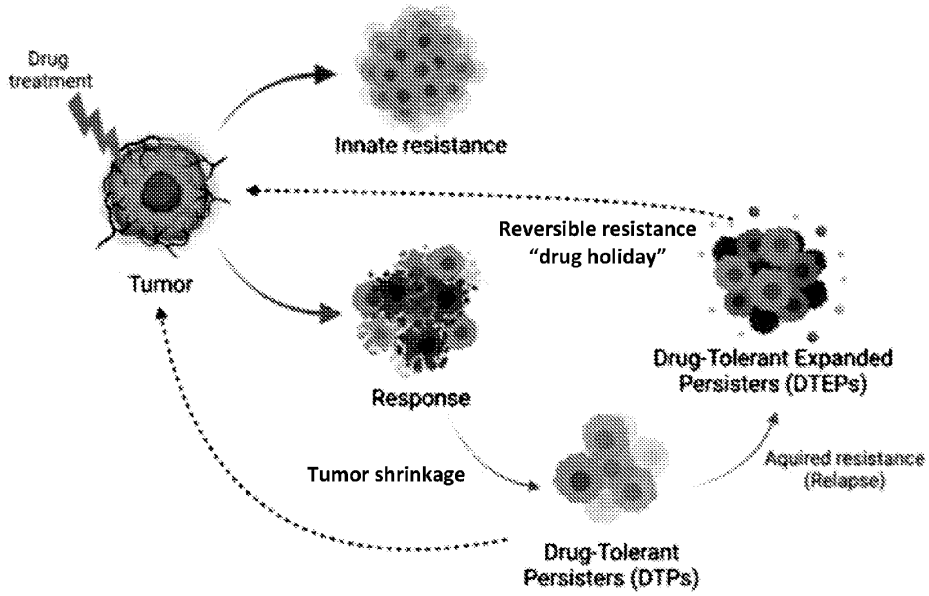

Drug treatment
Innate resistance
Tumor
Reversible resistance "drug holiday"
Drug-Tolerant Expanded Persisters (DTEPs)
Response
Tumor shrinkage
Aquired resistance (Relapse)
Drug-Tolerant Persisters (DTPs)

FIG. 1A

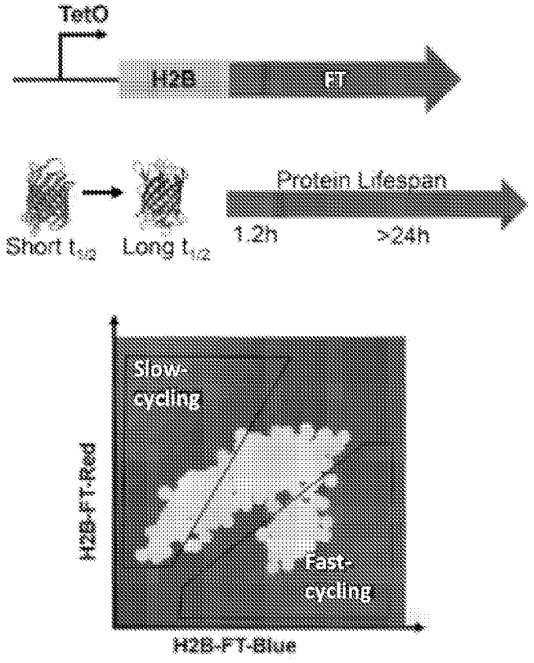

TetO
H2B
FT
Protein Lifespan
Short t1/2
Long t1/2
1.2h
>24h
Slow-cycling
Fast-cycling
H2B-FT-Red
H2B-FT-Blue

FIG. 1B

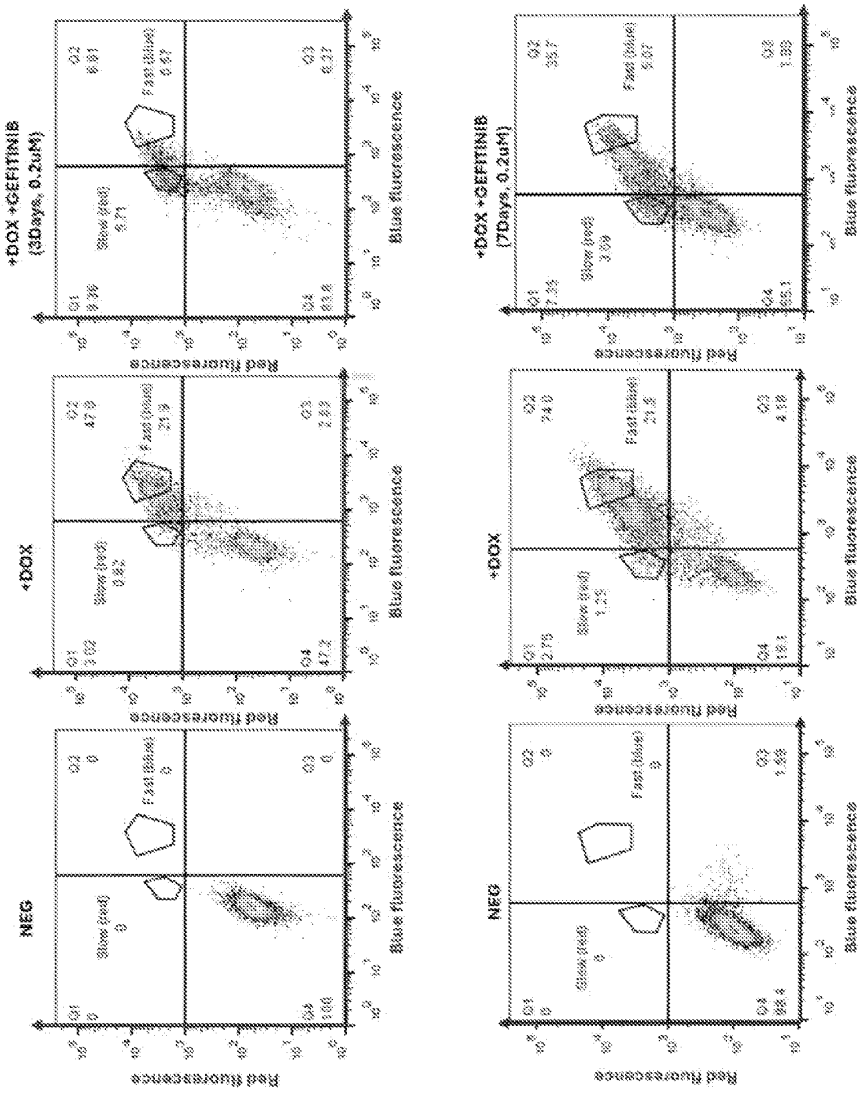

NEG
+DOX
+DOX +CEFITINIB (3Days, 0.2uM)
+DOX +CEFITINIB (7Days, 0.2uM)
Red fluorescence
Blue fluorescence
Slow (red)
Fast (blue)

FIG. 1C

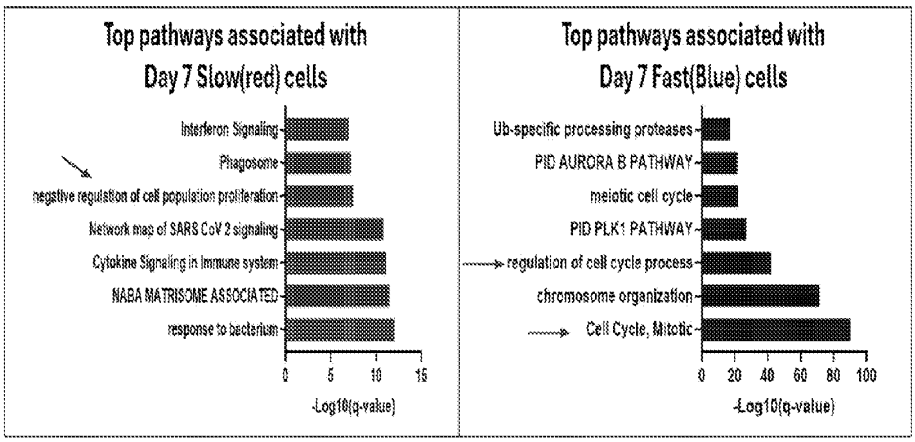

Top pathways associated with
Day 7 Slow(red) cells
Interferon Signaling
Phagosome
negative regulation of cell population proliferation
Network map of SARS CoV 2 signaling
Cytokine Signaling in Immune system
NABA MATRISOME ASSOCIATED
response to bacterium
0
5
10
15
-Log10(q-value)
Top pathways associated with
Day 7 Fast(Blue) cells
Ub-specific processing proteases
PID AURORA B PATHWAY
meiotic cell cycle
PID PLK1 PATHWAY
regulation of cell cycle process
chromosome organization
Cell Cycle, Mitotic
0
20
40
60
80
100
-Log10(q-value)

FIG. 1D

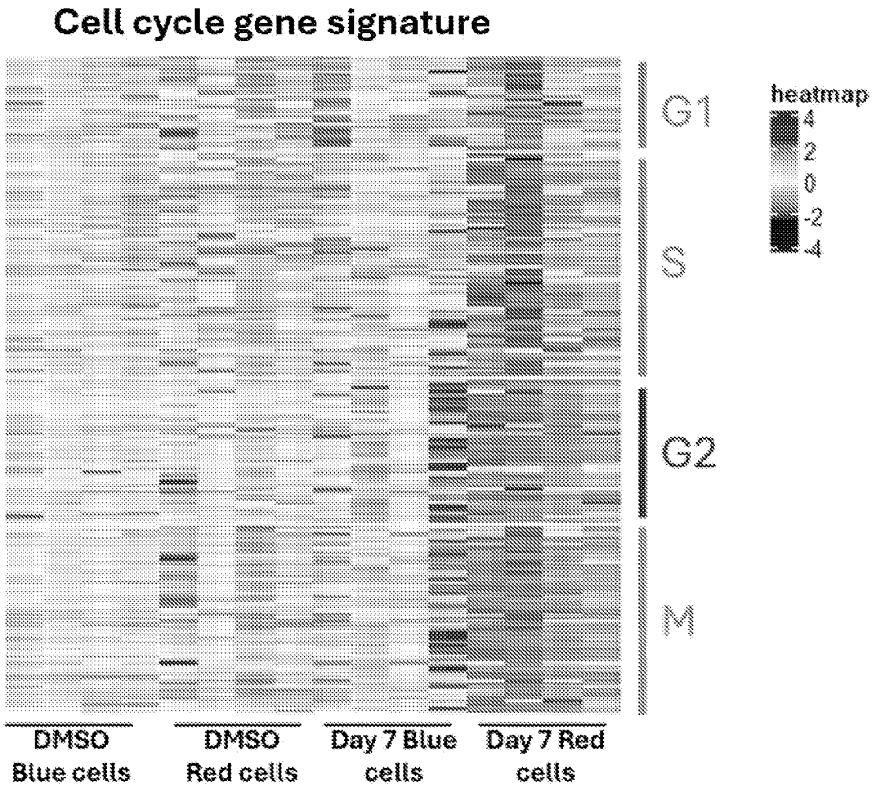

Cell cycle gene signature
G1
S
G2
M
heatmap
4
2
0
-2
-4
DMSO
Blue cells
DMSO
Red cells
Day 7 Blue
cells
Day 7 Red
cells

FIG. 1E

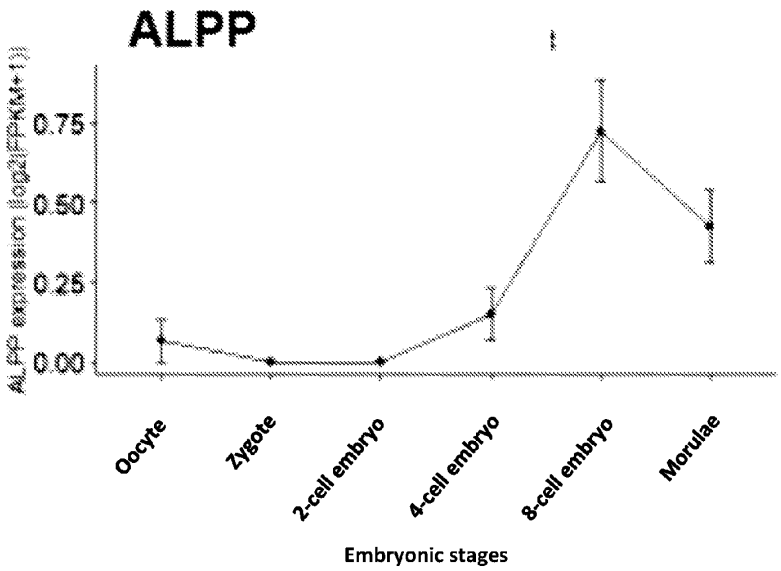

ALPP
ALPP expression (log2(FPKM+1))
0.75
0.50
0.25
0.00
Oocyte
Zygote
2-cell embryo
4-cell embryo
8-cell embryo
Morulae
Embryonic stages

FIG. 2A

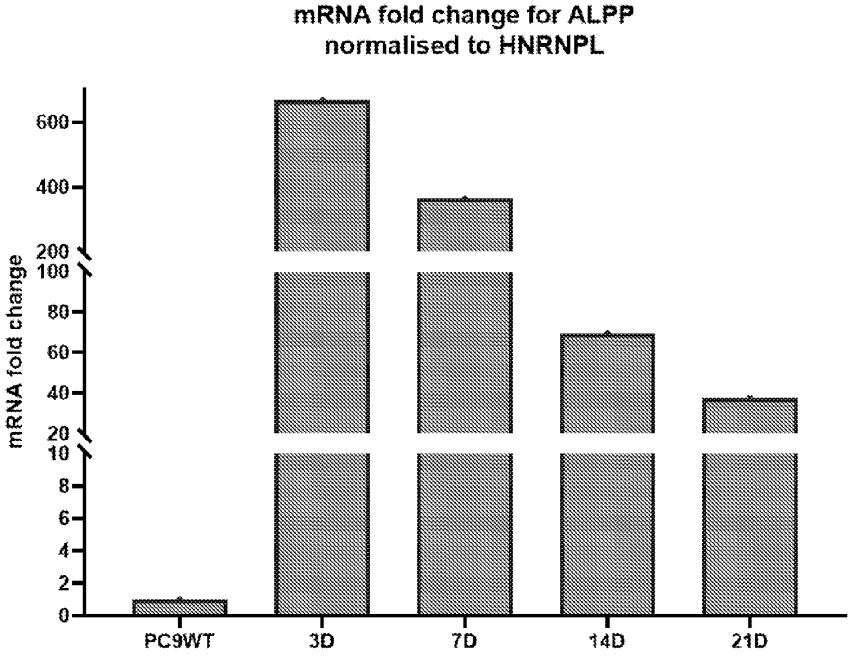

mRNA fold change for ALPP
normalised to HNRNPL
mRNA fold change
600
400
200
100
80
60
40
20
10
8
6
4
2
0
PC9WT
3D
7D
14D
21D

FIG. 2B

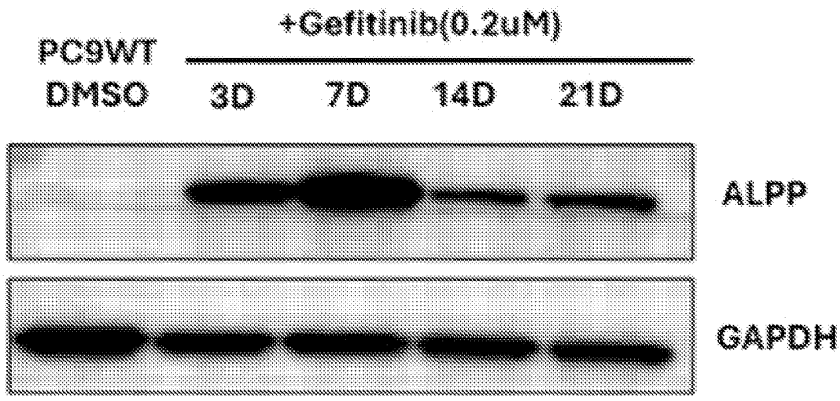

PC9WT
+Gefitinib(0.2uM)
DMSO
3D
7D
14D
21D
ALPP
GAPDH

FIG. 2C

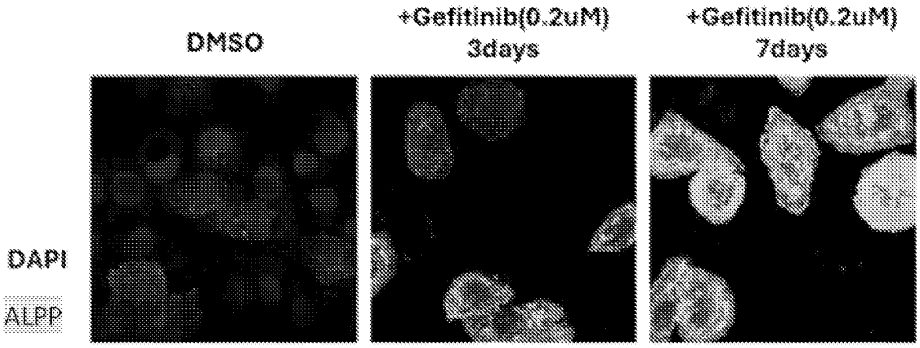

DMSO
+Gefitinib(0.2uM)
3days
+Gefitinib(0.2uM)
7days
DAPI
ALPP

FIG. 2D

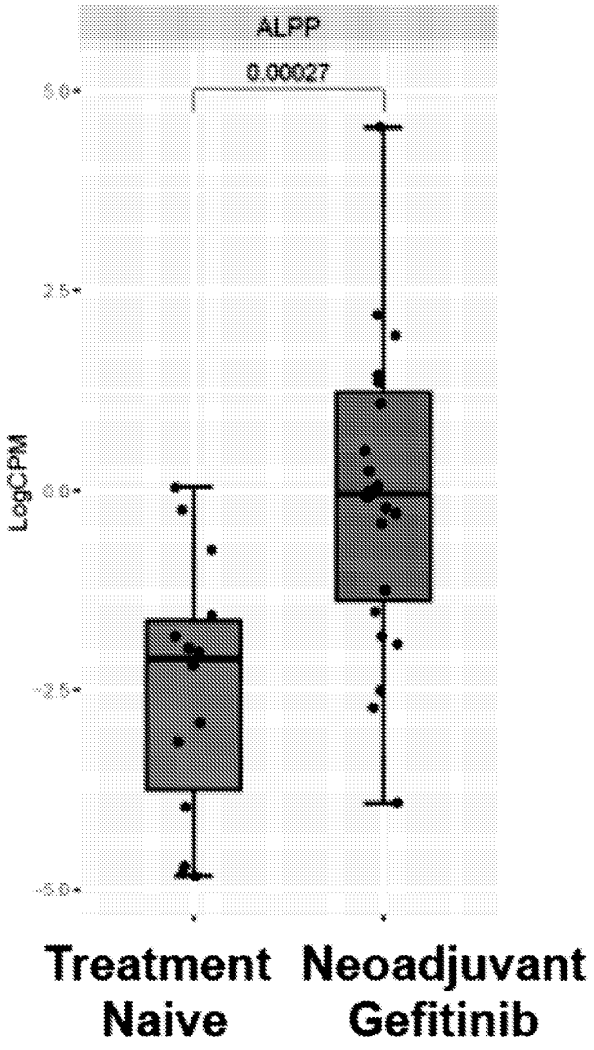

ALPP
0.00027
LogCPM
Treatment Naive
Neoadjuvant Gefitinib

FIG. 2E

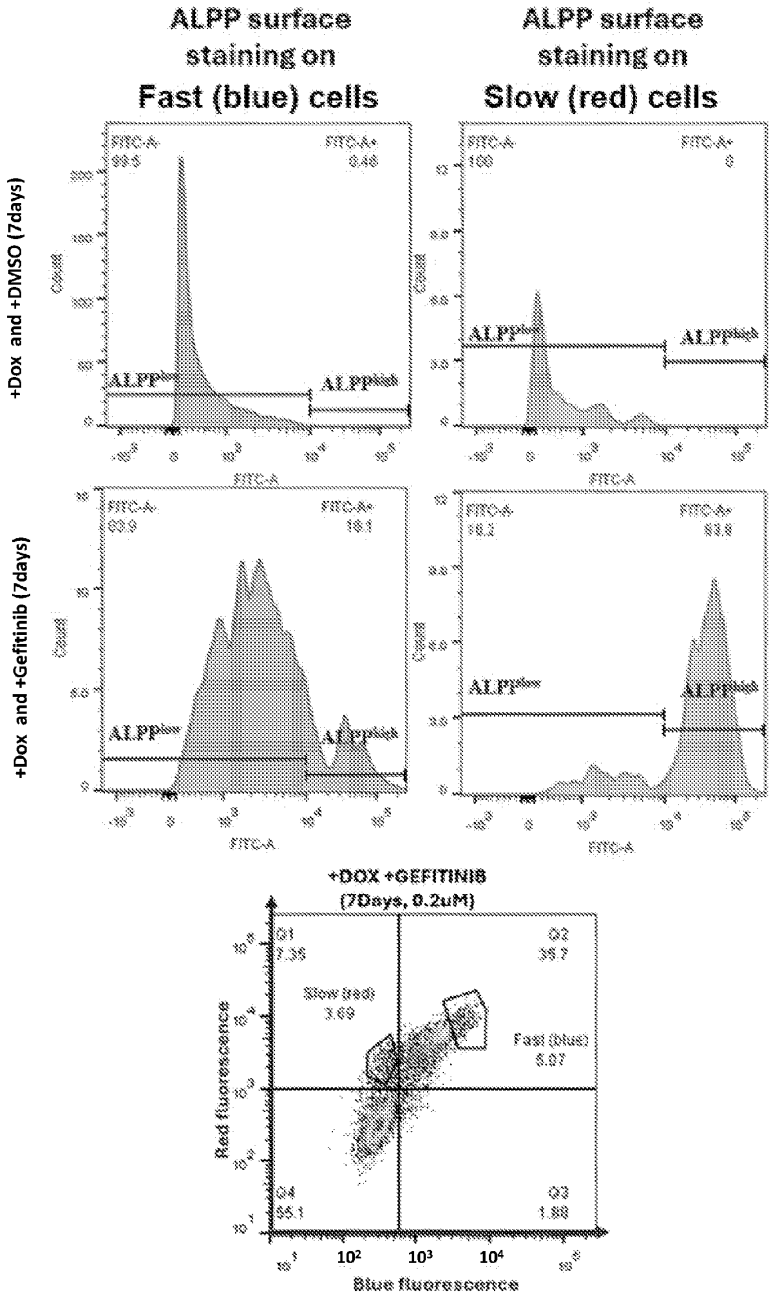

ALPP surface staining on Fast (blue) cells
ALPP surface staining on Slow (red) cells
+Dox and +DMSO (7days)
+Dox and +Gefitinib (7days)
Count
FITC-A
ALPP^low
ALPP^high
+DOX +GEFITINIB
(7Days, 0.2uM)
Q1
7.35
Q2
36.7
Slow (red)
3.69
Fast (blue)
5.07
Q4
55.1
Q3
1.88
Red fluorescence
Blue fluorescence

FIG. 2F

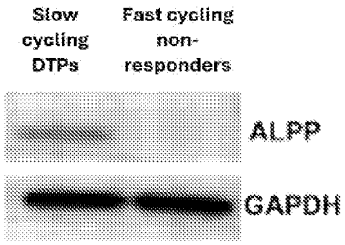

Slow cycling DTPs
Fast cycling non-responders
ALPP
GAPDH

FIG. 2G

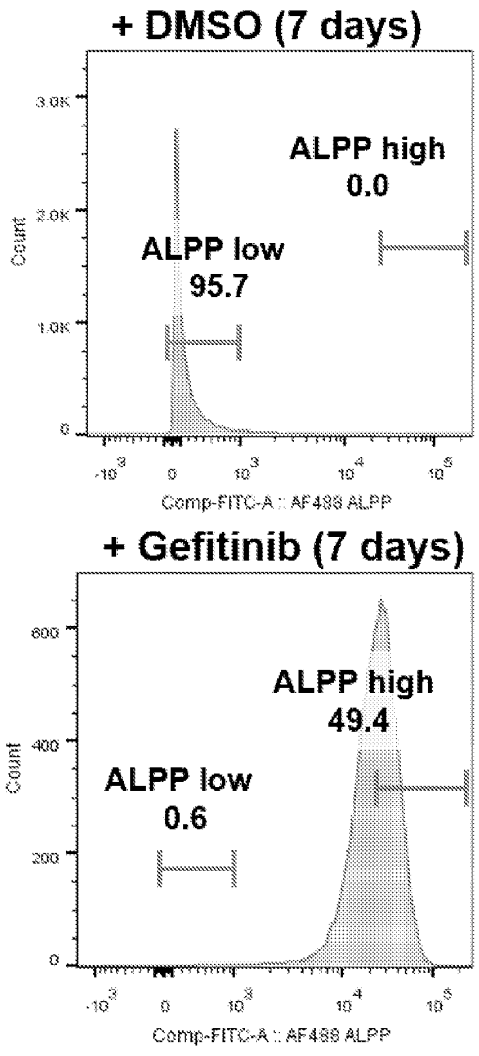

+ DMSO (7 days)
ALPP high
0.0
ALPP low
95.7
Count
Comp-FITC-A :: AF488 ALPP
+ Gefitinib (7 days)
ALPP high
49.4
ALPP low
0.6
Count
Comp-FITC-A :: AF488 ALPP

FIG. 3A

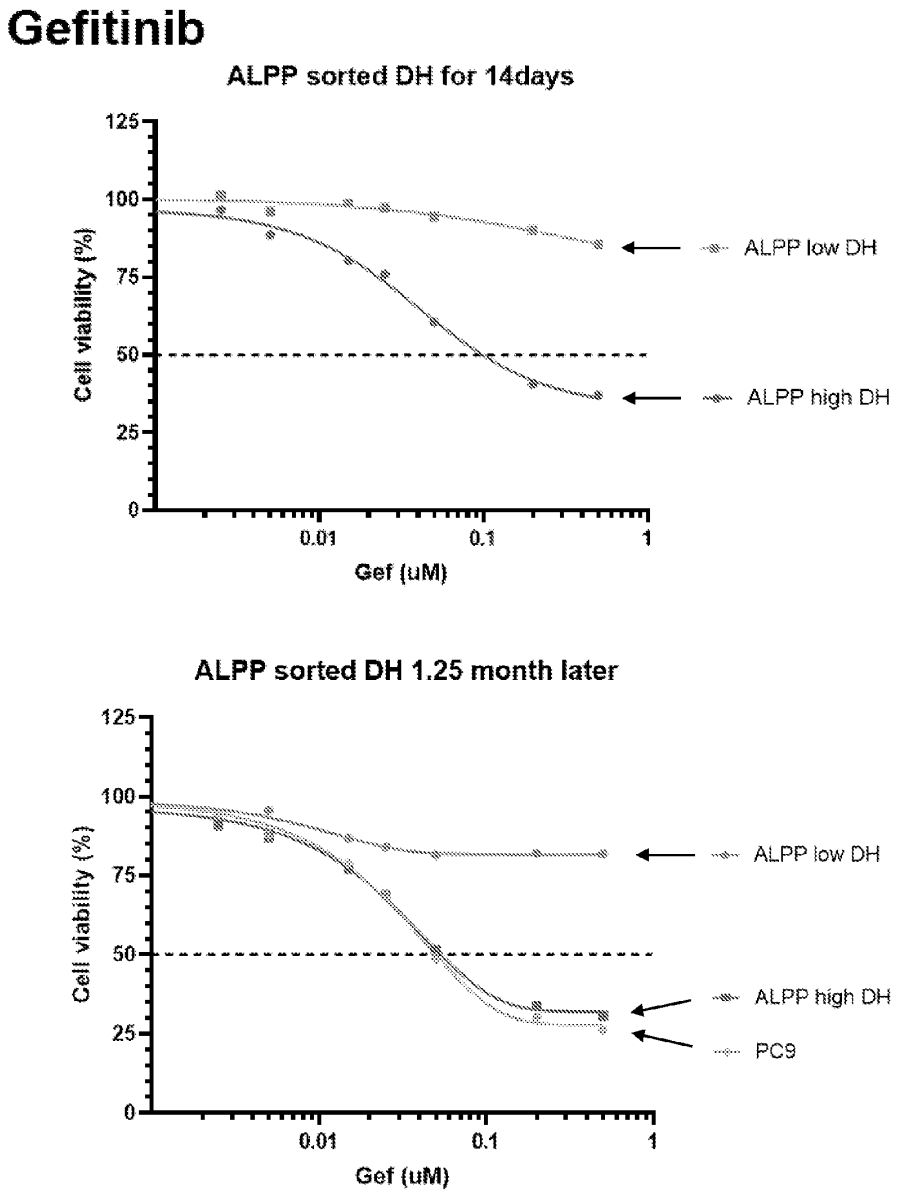

Gefitinib
ALPP sorted DH for 14days
Cell viability (%)
125
100
75
50
25
0
0.01
0.1
1
Gef (uM)
ALPP low DH
ALPP high DH
ALPP sorted DH 1.25 month later
Cell viability (%)
125
100
75
50
25
0
0.01
0.1
1
Gef (uM)
ALPP low DH
ALPP high DH
PC9

FIG. 3B

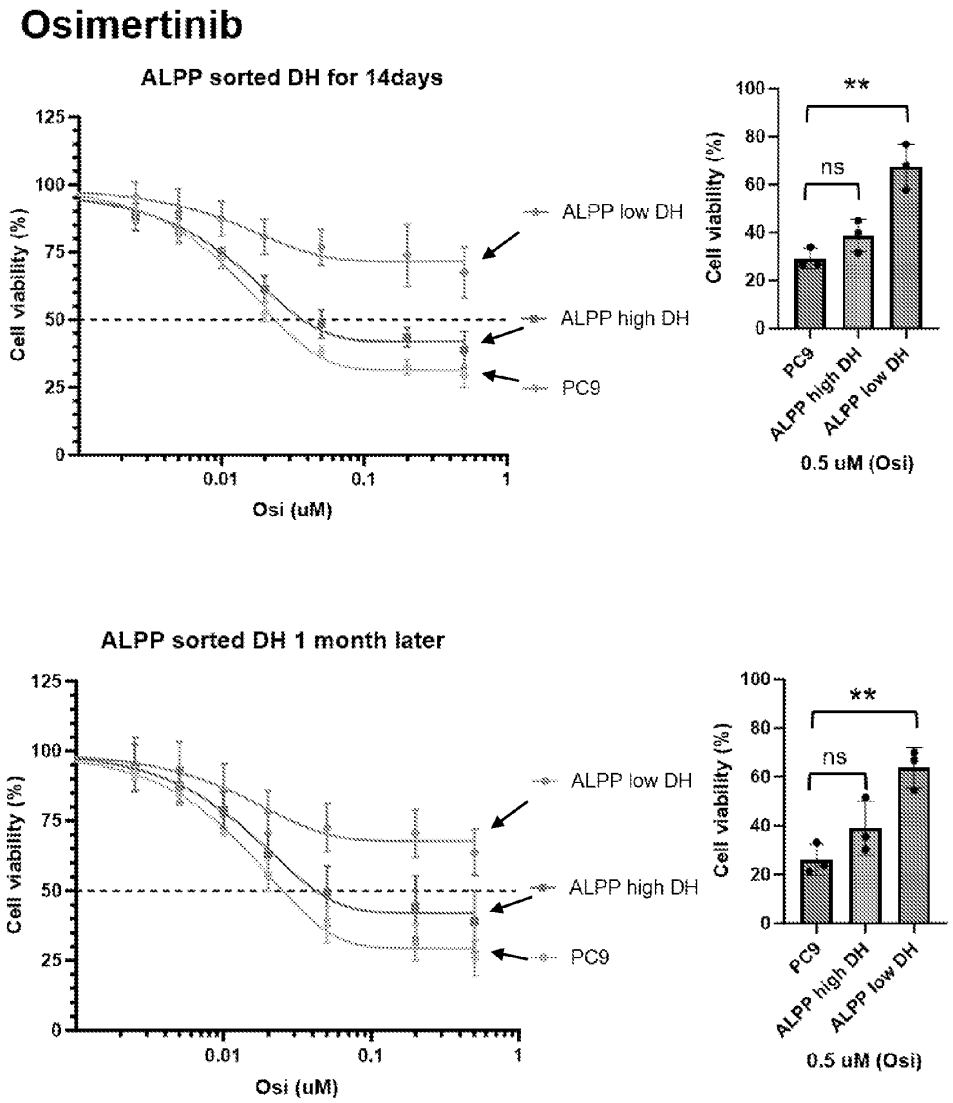


FIG. 3B (continued)

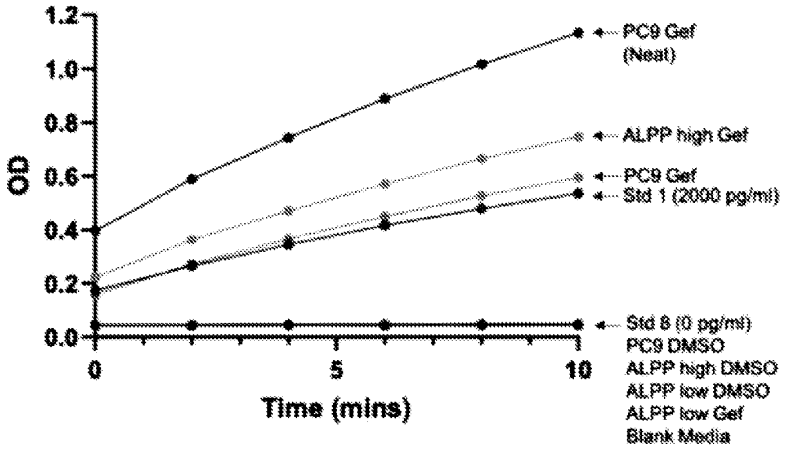

1.2
1.0
0.8
0.6
0.4
0.2
0.0
OD
0
5
10
Time (mins)
PC9 Gef (Neat)
ALPP high Gef
PC9 Gef
Std 1 (2000 pg/ml)
Std 8 (0 pg/ml)
PC9 DMSO
ALPP high DMSO
ALPP low DMSO
ALPP low Gef
Blank Media

FIG. 3C

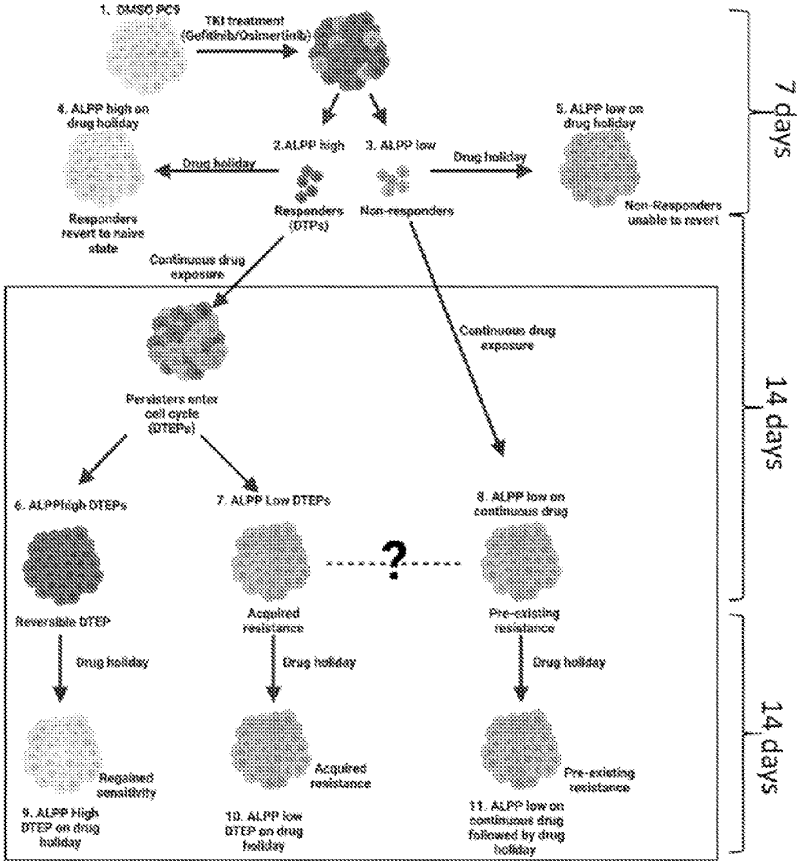

1. DMSO PC9
TKI treatment (Gefitinib/Osimertinib)
4. ALPP high on drug holiday
2.ALPP high
3. ALPP low
5. ALPP low on drug holiday
Drug holiday
Drug holiday
Responders revert to naive state
Responders (DTPs)
Non-responders
Non-Responders unable to revert
7 days
Continuous drug exposure
Continuous drug exposure
Persisters enter cell cycle (DTEPs)
6. ALPPHigh DTEPs
7. ALPP Low DTEPs
8. ALPP low on continuous drug
?
Reversible DTEP
Acquired resistance
Pre-existing resistance
14 days
Drug holiday
Drug holiday
Drug holiday
Regained sensitivity
Acquired resistance
Pre-existing resistance
9. ALPP High DTEP on drug holiday
10. ALPP low DTEP on drug holiday
11. ALPP low on continuous drug followed by drug holiday
14 days

FIG. 4A

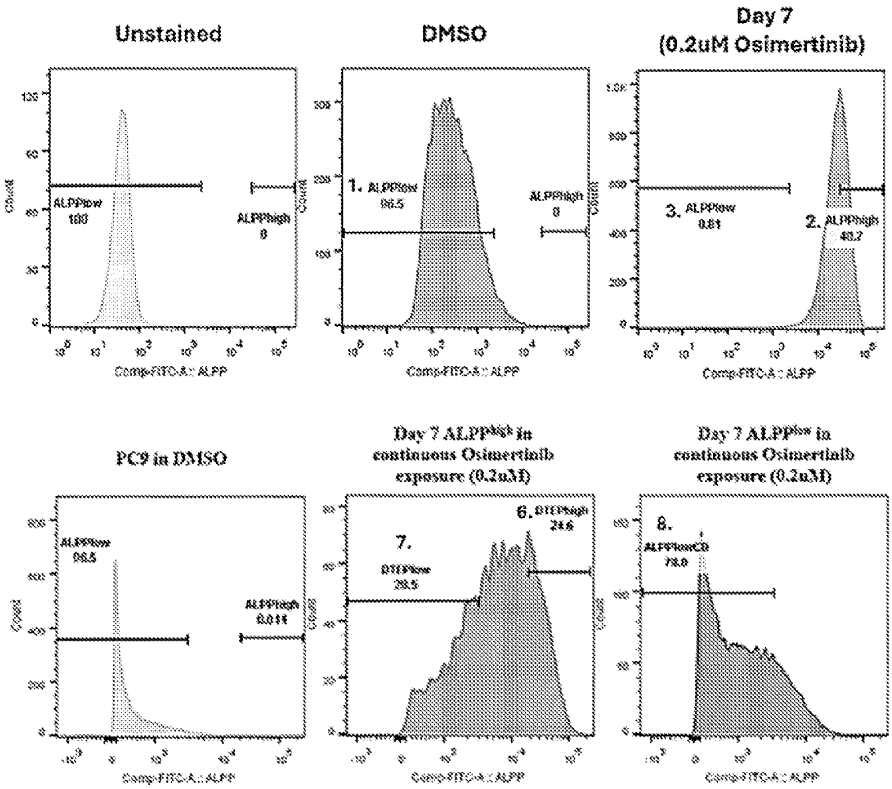


FIG. 4A (continued)

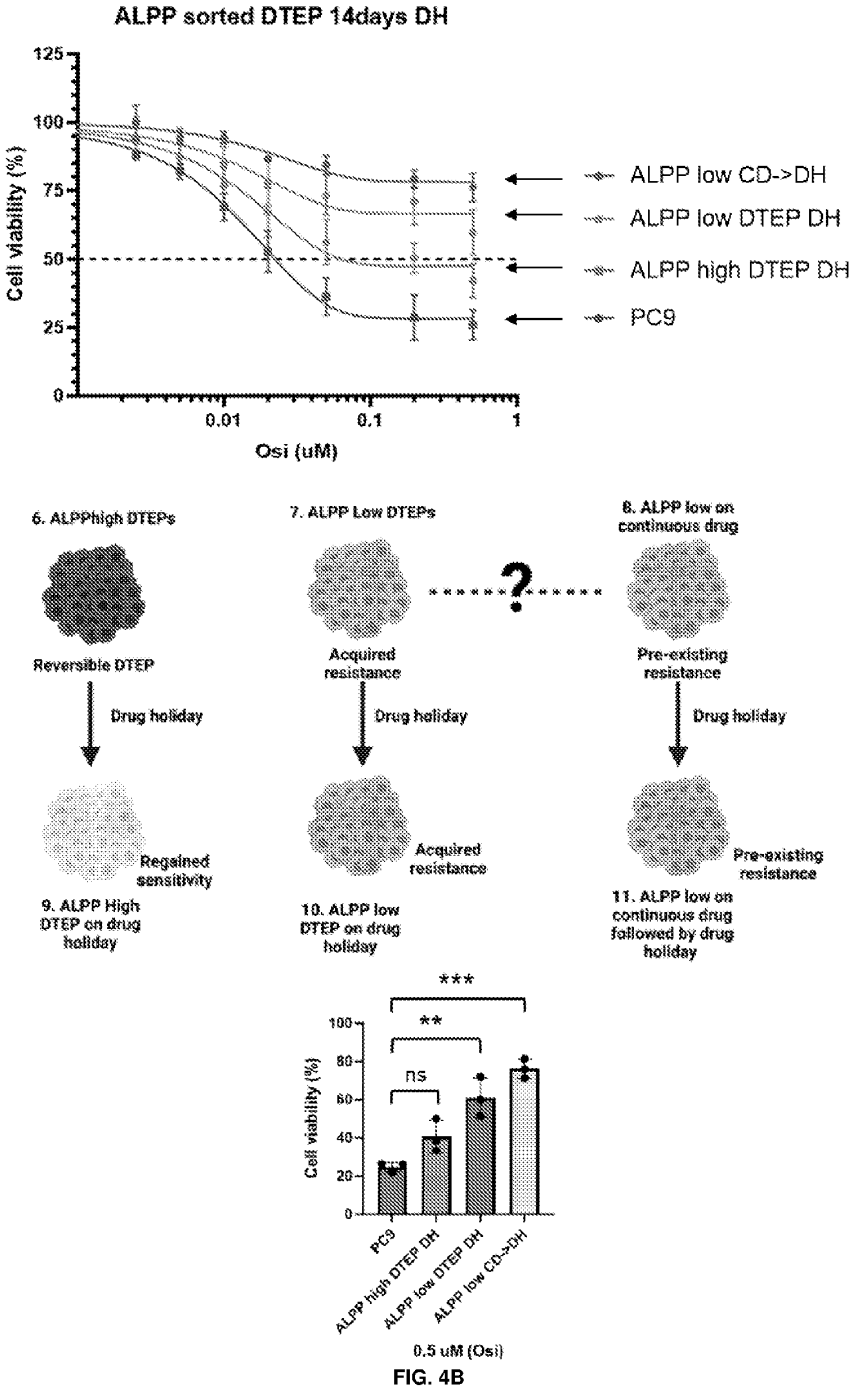

ALPP sorted DTEP 14days DH
Cell viability (%)
125
100
75
50
25
0
0.01
0.1
1
Osi (uM)
ALPP low CD->DH
ALPP low DTEP DH
ALPP high DTEP DH
PC9
6. ALPPhigh DTEPs
Reversible DTEP
Drug holiday
Regained sensitivity
9. ALPP High DTEP on drug holiday
7. ALPP Low DTEPs
Acquired resistance
Drug holiday
Acquired resistance
10. ALPP low DTEP on drug holiday
8. ALPP low on continuous drug
Pre-existing resistance
Drug holiday
Pre-existing resistance
11. ALPP low on continuous drug followed by drug holiday
***
**
ns
Cell viability (%)
100
80
60
40
20
0
PC9
ALPP high DTEP DH
ALPP low DTEP DH
ALPP low CD->DH
0.5 uM (Osi)

FIG. 4B

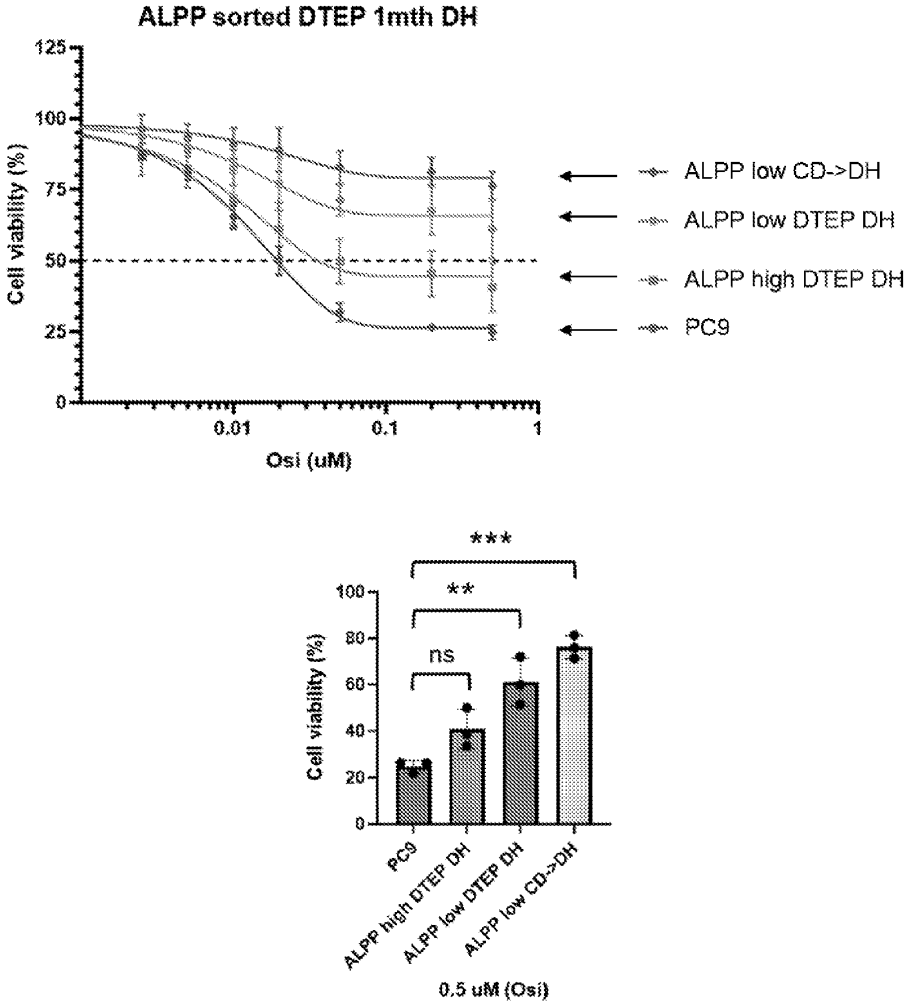

ALPP sorted DTEP 1mth DH
Cell viability (%)
125
100
75
50
25
0
0.01
0.1
1
Osi (uM)
ALPP low CD->DH
ALPP low DTEP DH
ALPP high DTEP DH
PC9
***
**
ns
Cell viability (%)
100
80
60
40
20
0
PC9
ALPP high DTEP DH
ALPP low DTEP DH
ALPP low CD->DH
0.5 uM (Osi)

FIG. 4C

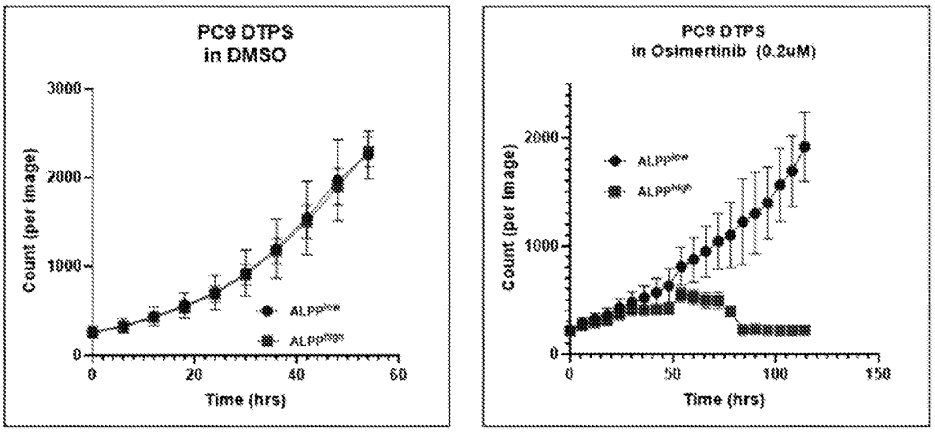

PC9 DTPS
in DMSO
Count (per image)
Time (hrs)
ALPPlow
ALPPhigh
PC9 DTPS
in Osimertinib (0.2uM)
Count (per image)
Time (hrs)
ALPPlow
ALPPhigh

FIG. 5A

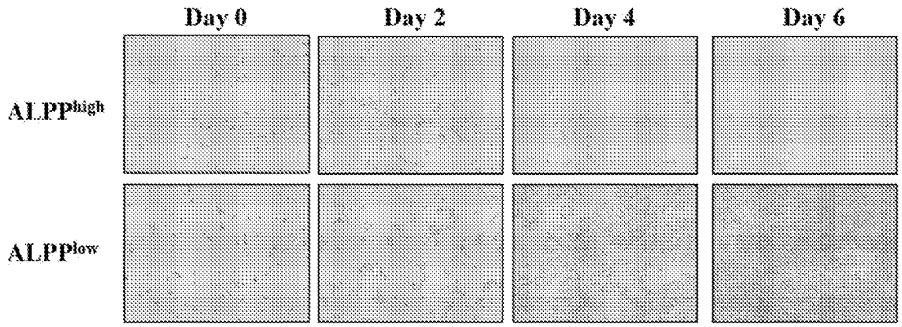

Day 0
Day 2
Day 4
Day 6
ALPPhigh
ALPPlow

FIG. 5B