Fig. 1

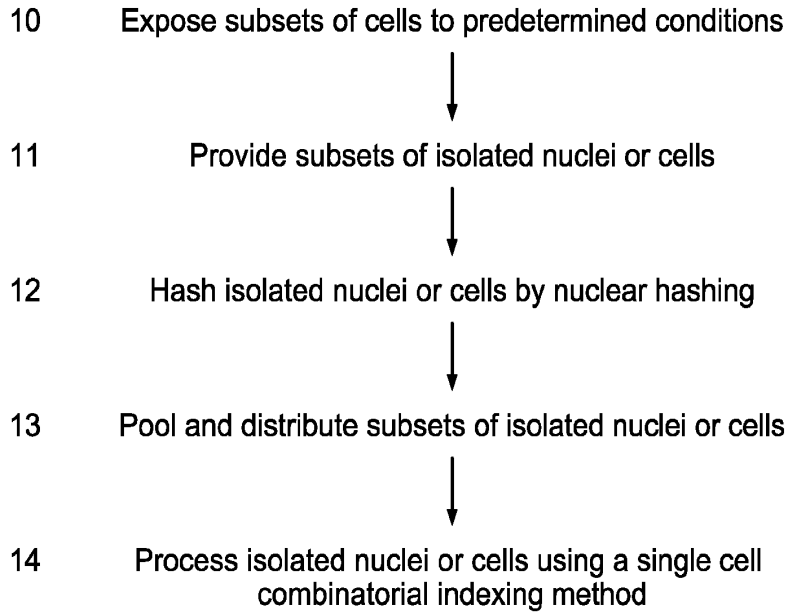

10
Expose subsets of cells to predetermined conditions
11
Provide subsets of isolated nuclei or cells
12
Hash isolated nuclei or cells by nuclear hashing
13
Pool and distribute subsets of isolated nuclei or cells
14
Process isolated nuclei or cells using a single cell combinatorial indexing method

Fig. 2

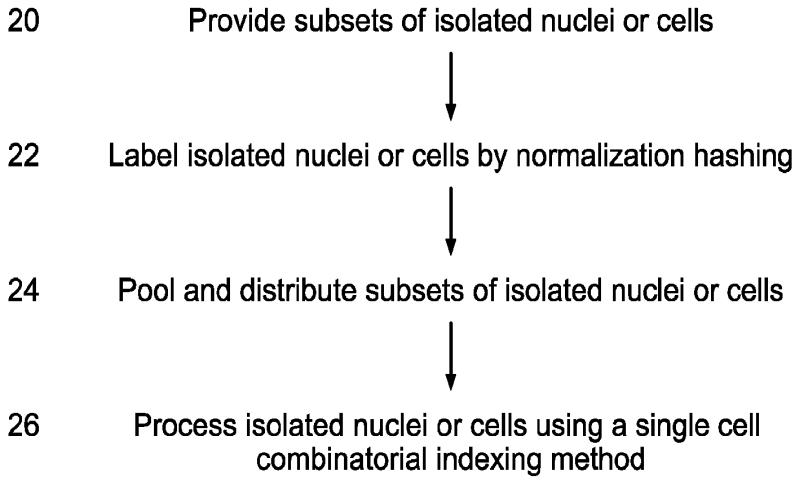

20
Provide subsets of isolated nuclei or cells
22
Label isolated nuclei or cells by normalization hashing
24
Pool and distribute subsets of isolated nuclei or cells
26
Process isolated nuclei or cells using a single cell combinatorial indexing method

Fig. 3

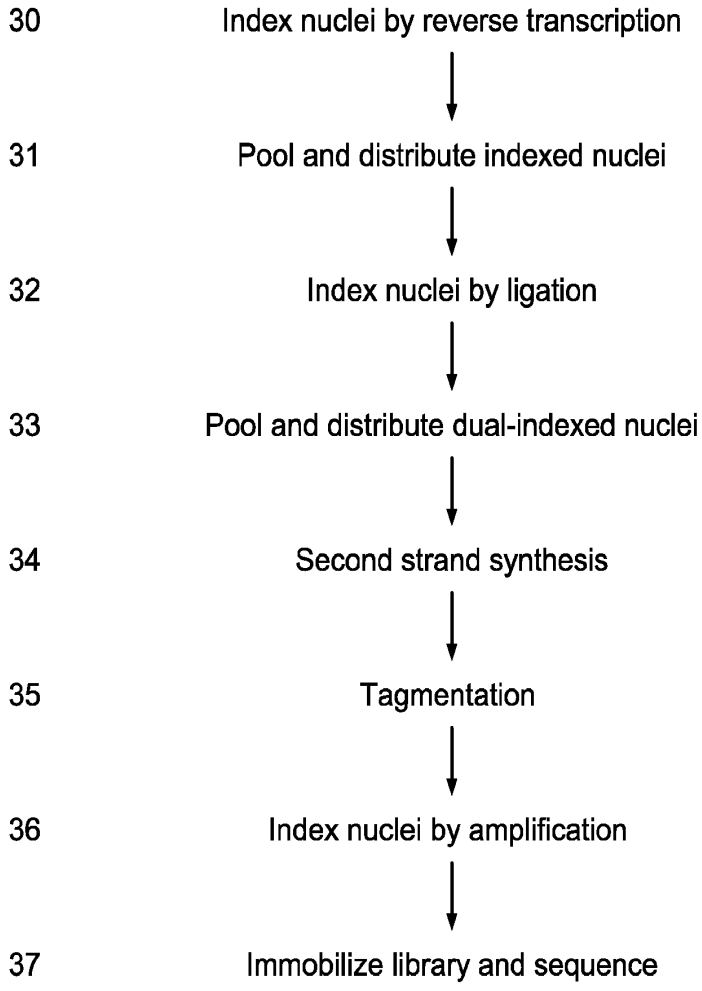
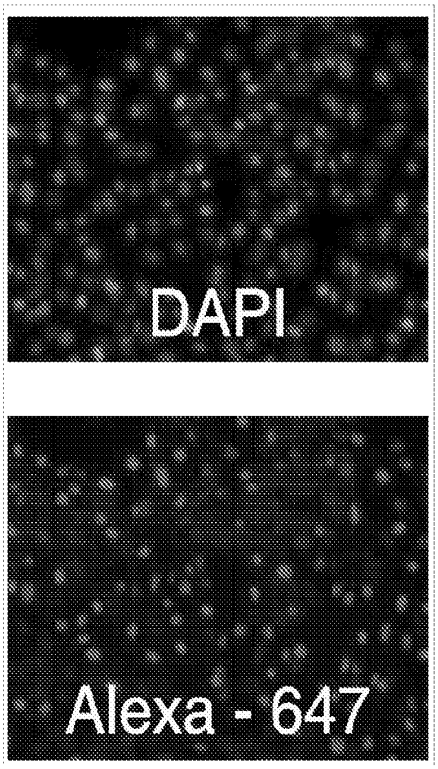

30
Index nuclei by reverse transcription
31
Pool and distribute indexed nuclei
32
Index nuclei by ligation
33
Pool and distribute dual-indexed nuclei
34
Second strand synthesis
35
Tagmentation
36
Index nuclei by amplification
37
Immobilize library and sequence

Fig. 4A

A



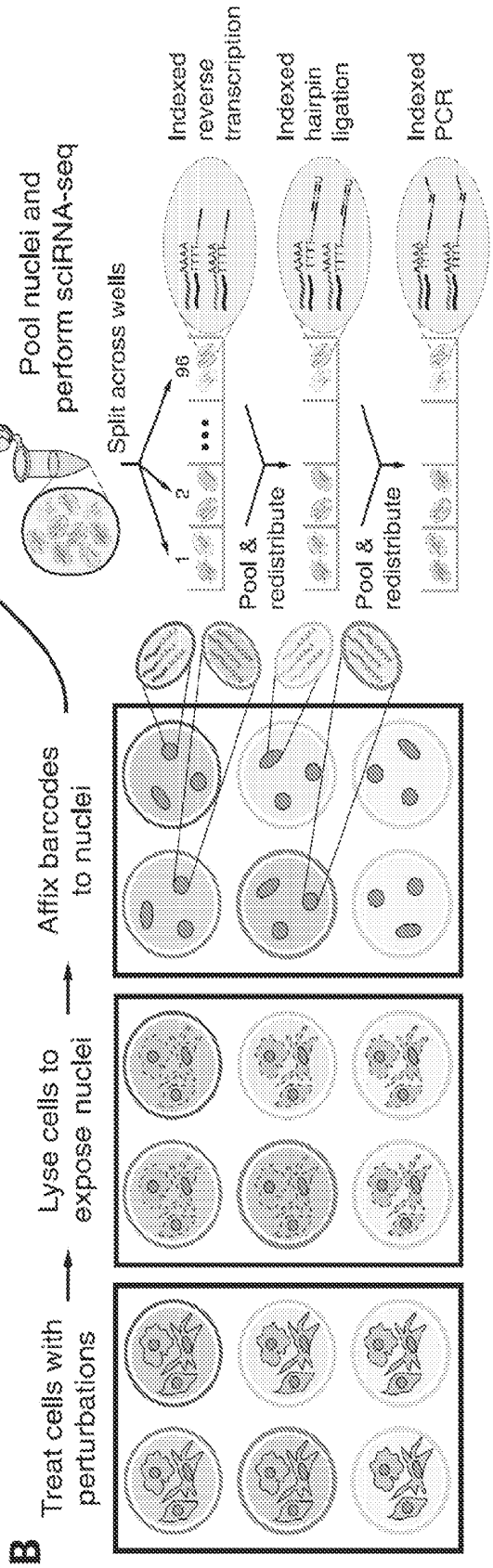

B
Treat cells with perturbations
Lyse cells to expose nuclei
Affix barcodes to nuclei
Pool nuclei and perform sciRNA-seq
Split across wells
1
2
96
Pool & redistribute
Pool & redistribute
Indexed reverse transcription
Indexed hairpin ligation
Indexed PCR

Fig. 4B

Fig. 4C

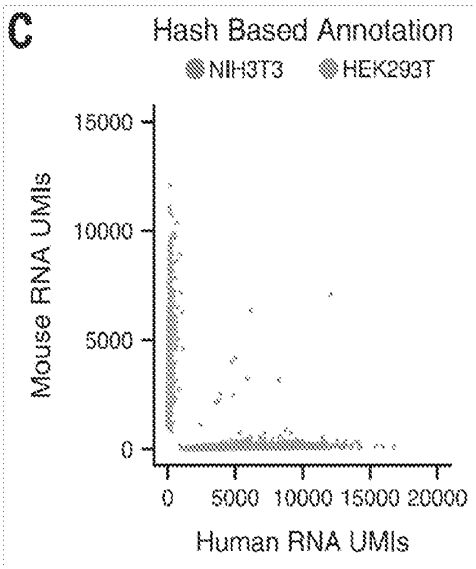


Fig. 4D

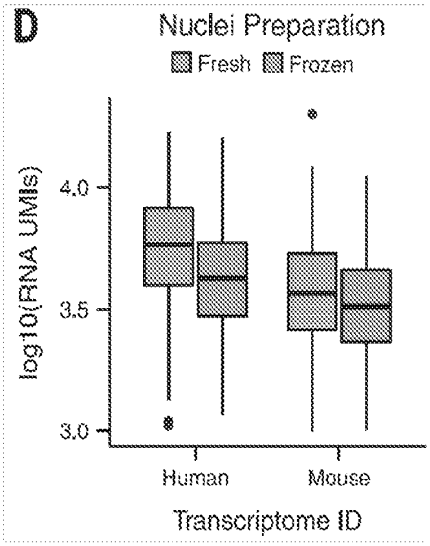


Fig. 4E

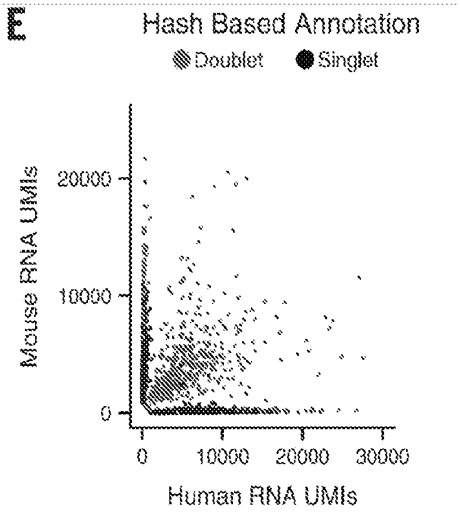


Fig. 5A

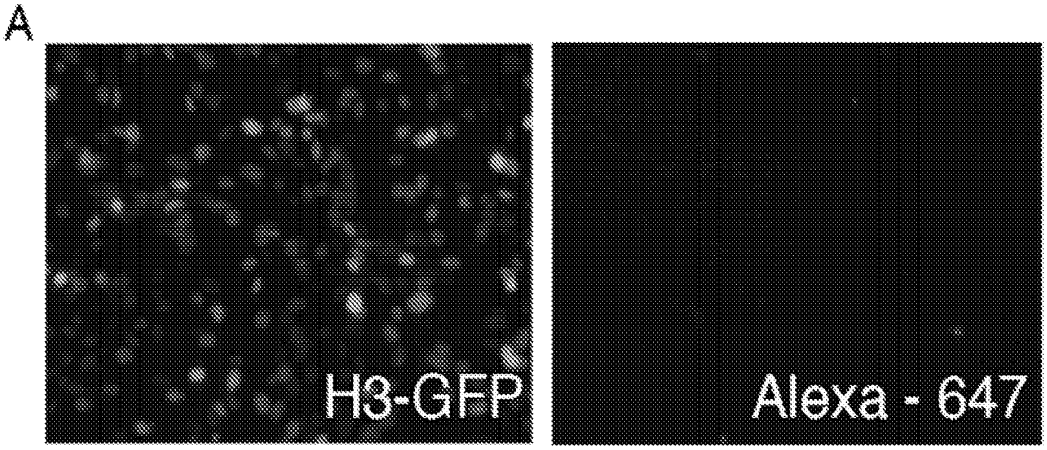


Fig. 5B

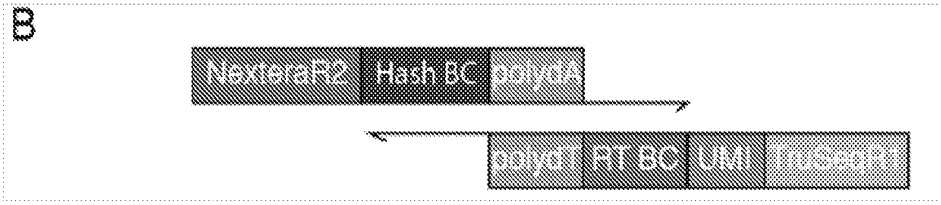


Fig. 5C

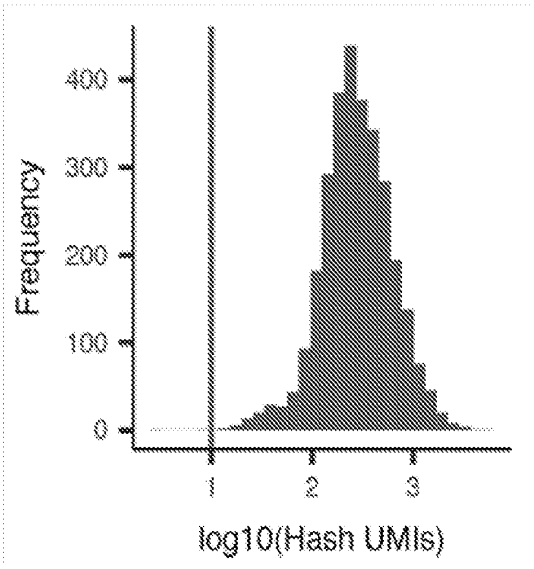


Fig. 5D

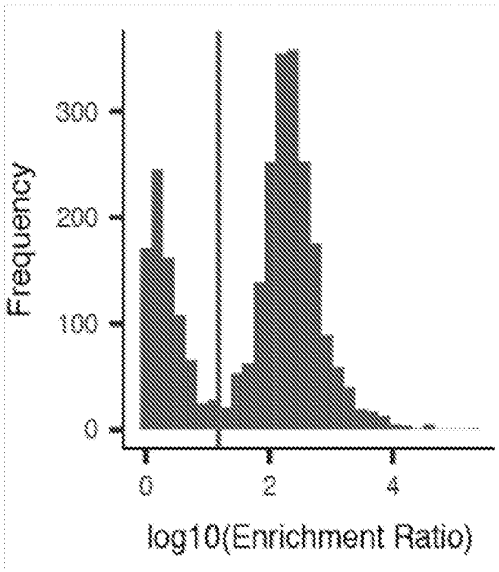


Fig. 5E

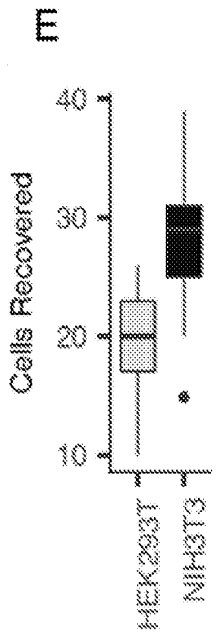


Fig. 5F

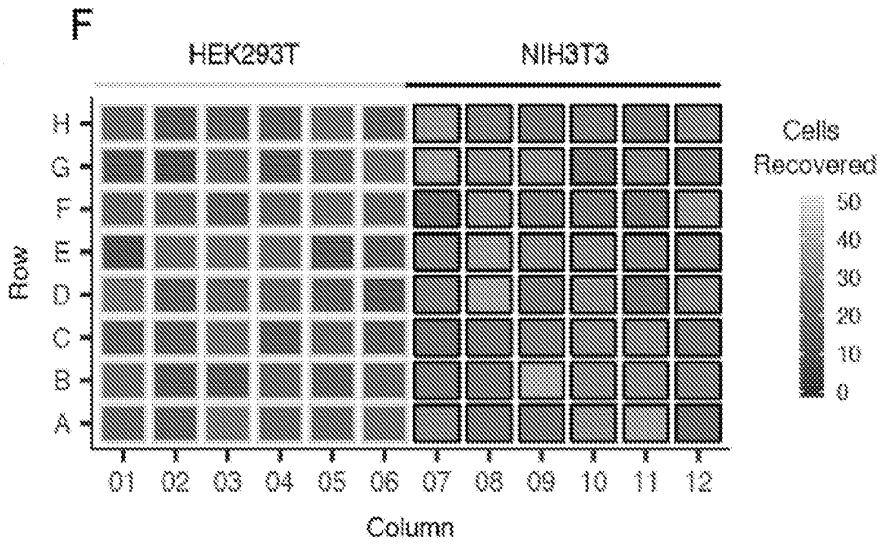


Fig. 5G

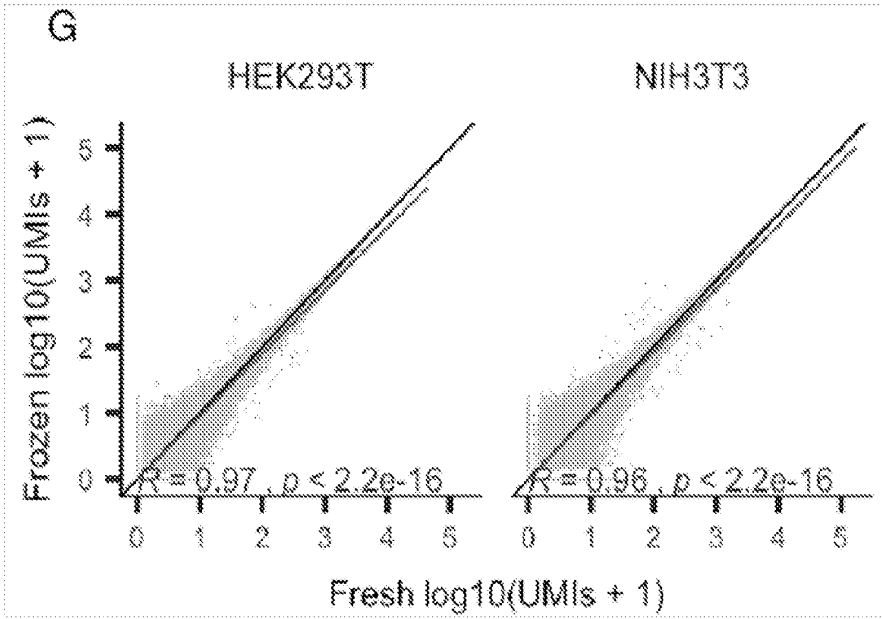


Fig. 5H

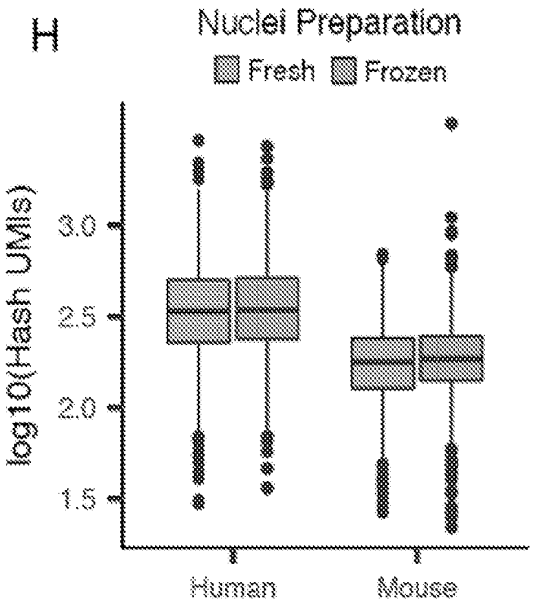


Fig. 5I

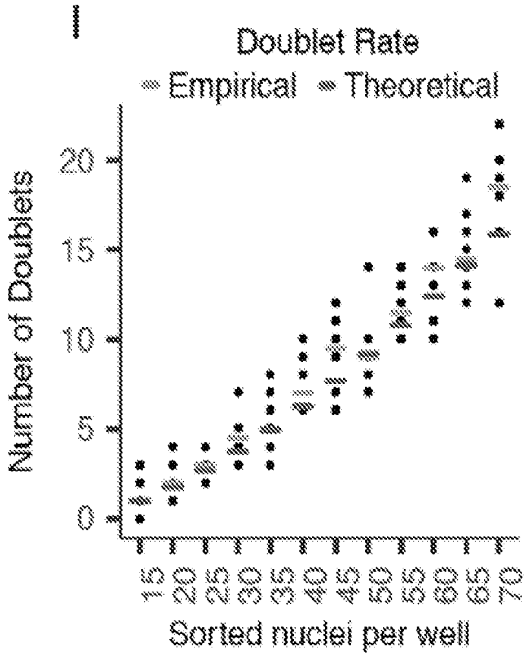


Fig. 5J

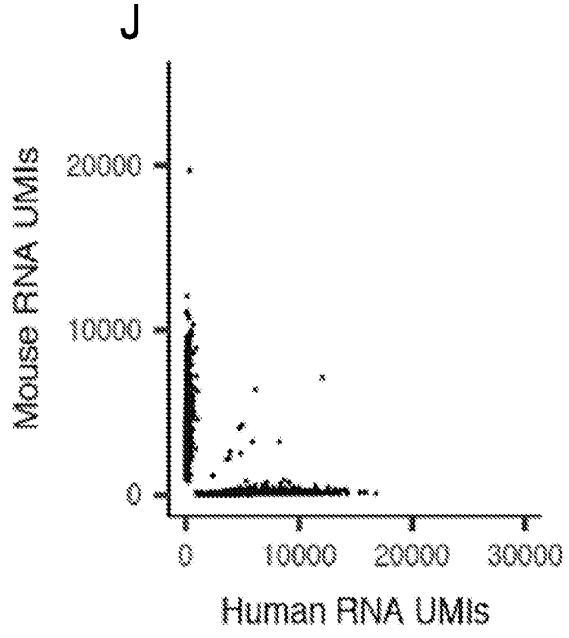


Fig. 5K

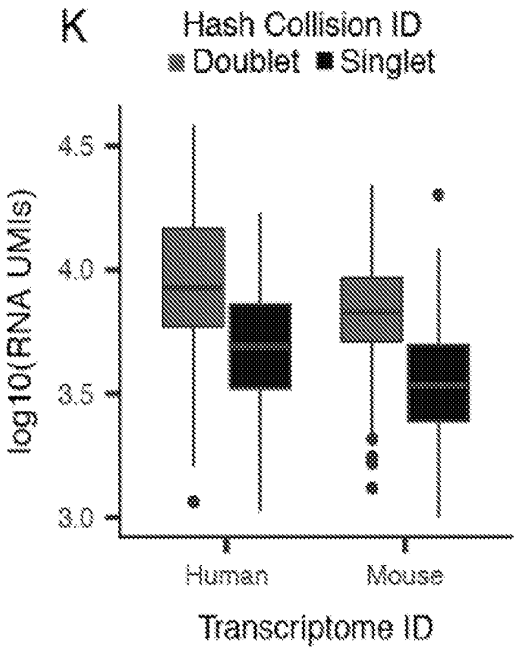


Fig. 6A

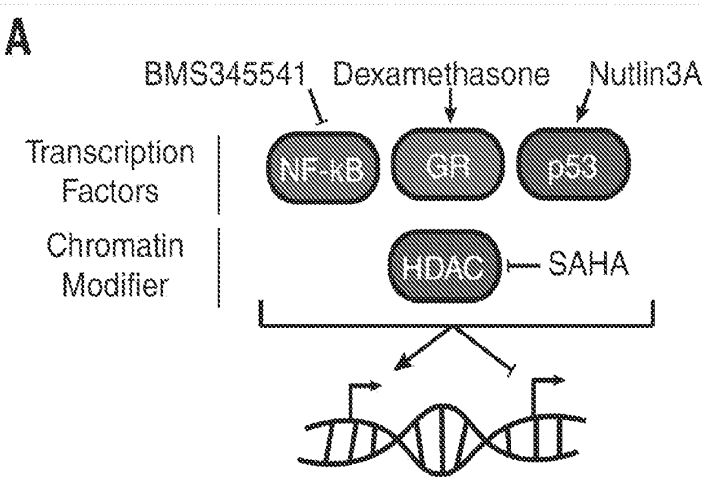


Fig. 6B

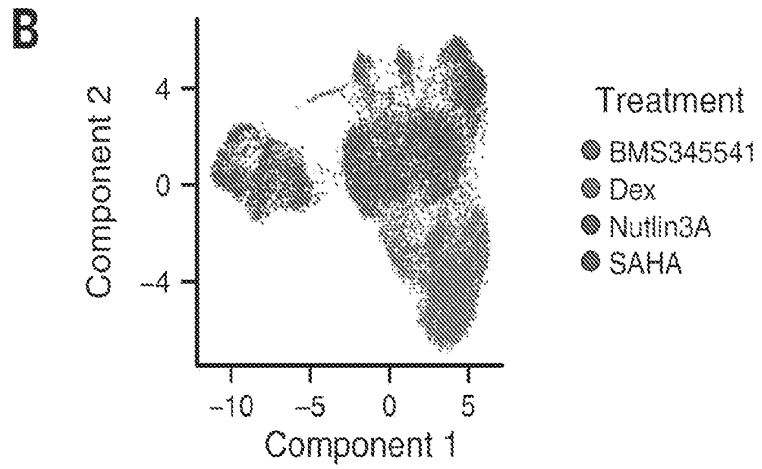


Fig. 6C

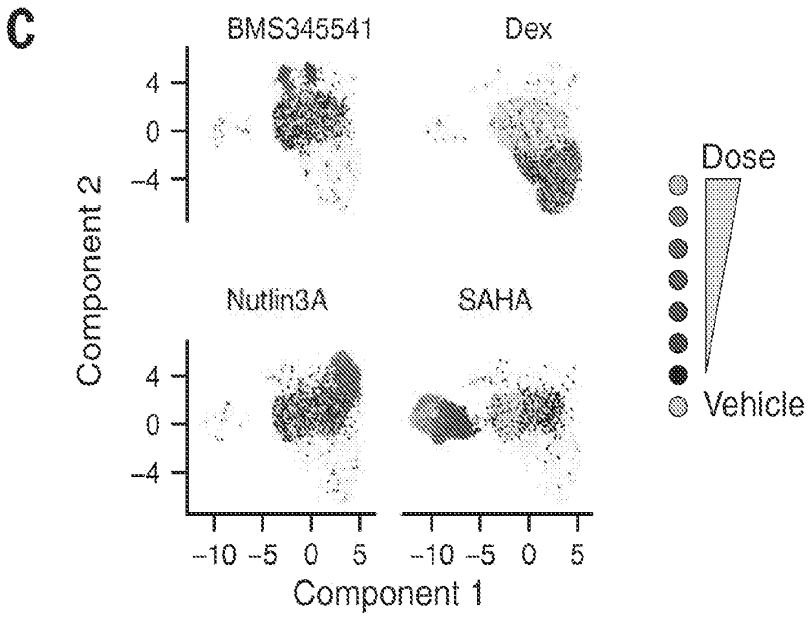


Fig. 6D

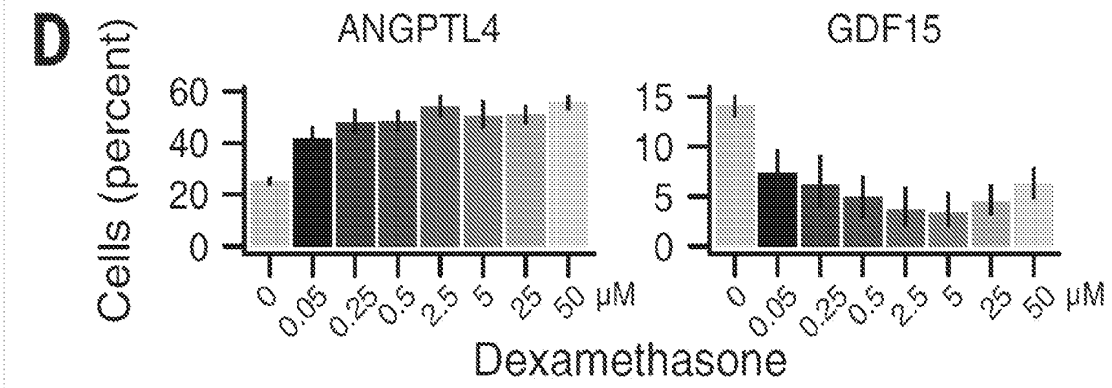

D
ANGPTL4
GDF15
Cells (percent)
60
40
20
0
15
10
5
0
0 0.05 0.25 0.5 2.5 5 25 50 μM
Dexamethasone

Fig. 6E

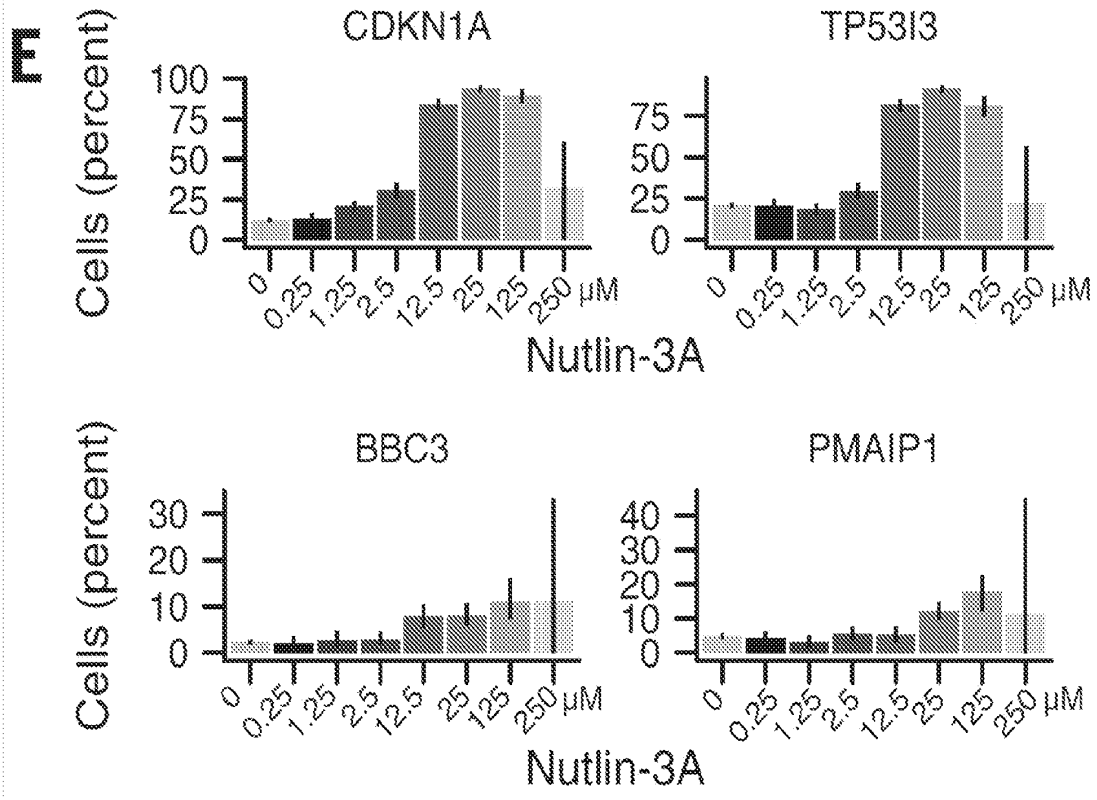

E
CDKN1A
TP53I3
Cells (percent)
100
75
50
25
0
75
50
25
0
0 0.25 1.25 2.5 12.5 25 125 250 μM
Nutlin-3A
BBC3
PMAIP1
30
20
10
0
40
30
20
10
0
0 0.25 1.25 2.5 12.5 25 125 250 μM
Nutlin-3A

Fig. 6F

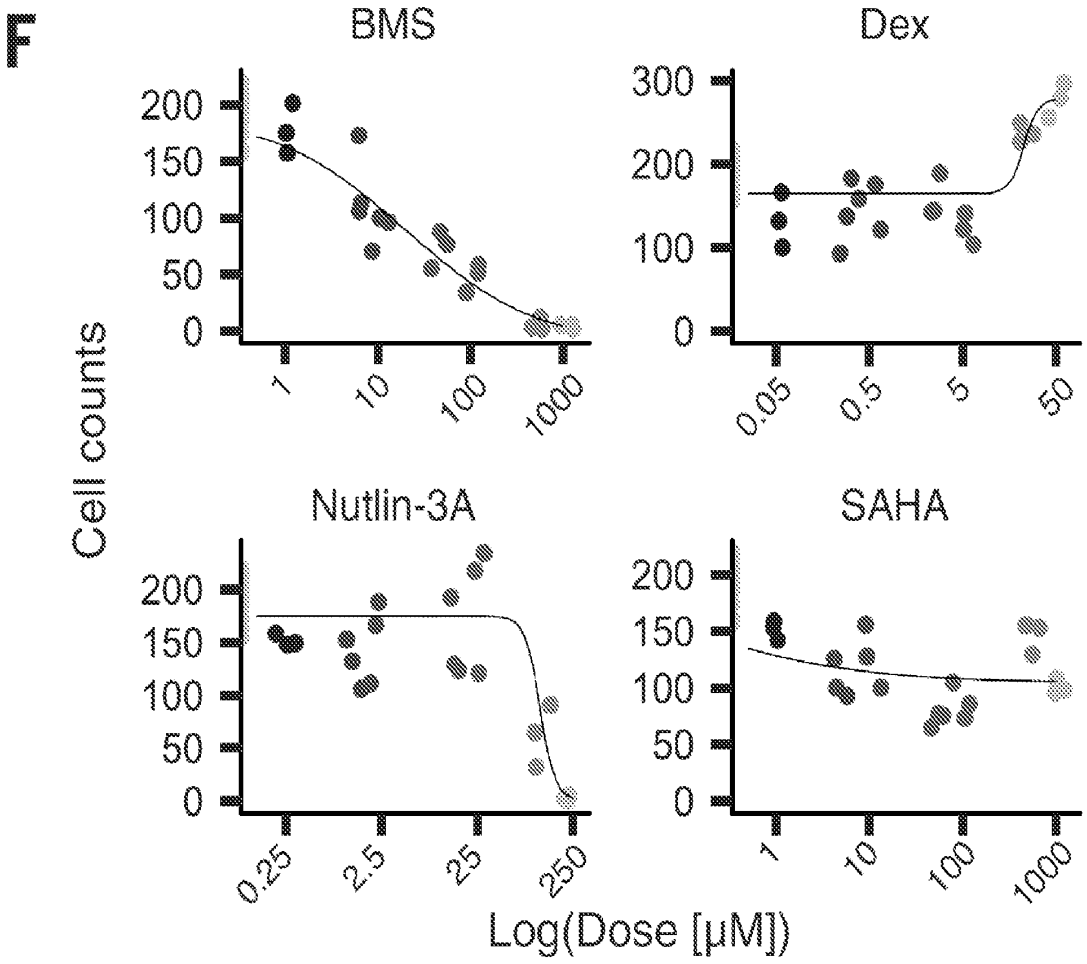

F
BMS
Dex
Nutlin-3A
SAHA
Cell counts
Log(Dose [μM])
200
150
100
50
0
300
1
10
100
1000
0.05
0.5
5
50
0.25
2.5
25
250

Fig. 7A

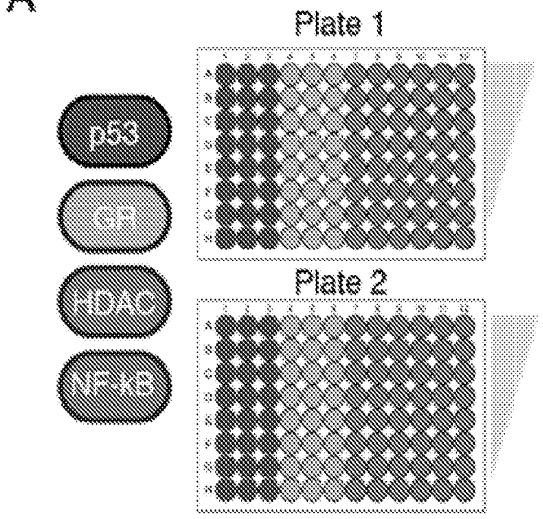


Fig. 7B

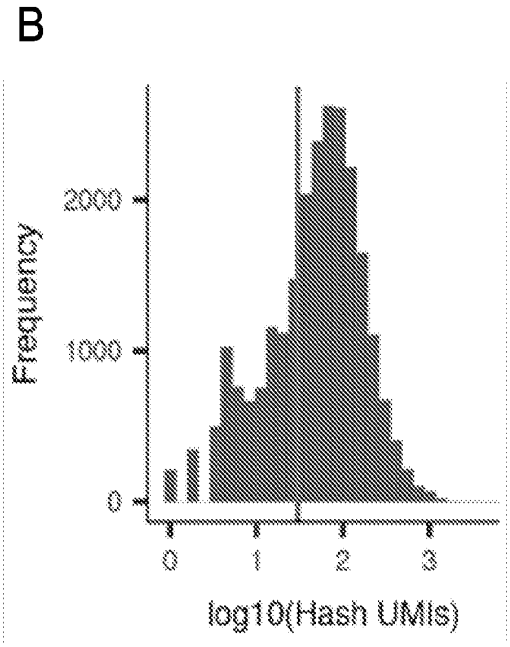


Fig. 7C

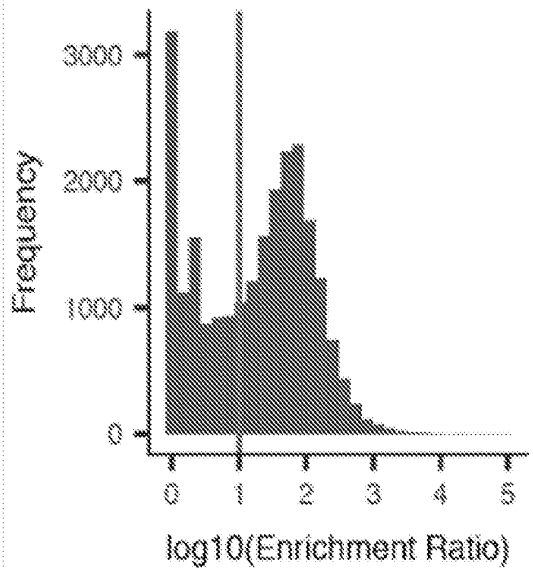


Fig. 7D

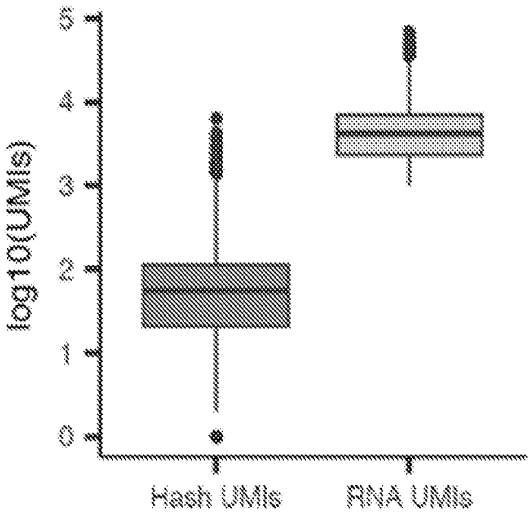


Fig. 7E

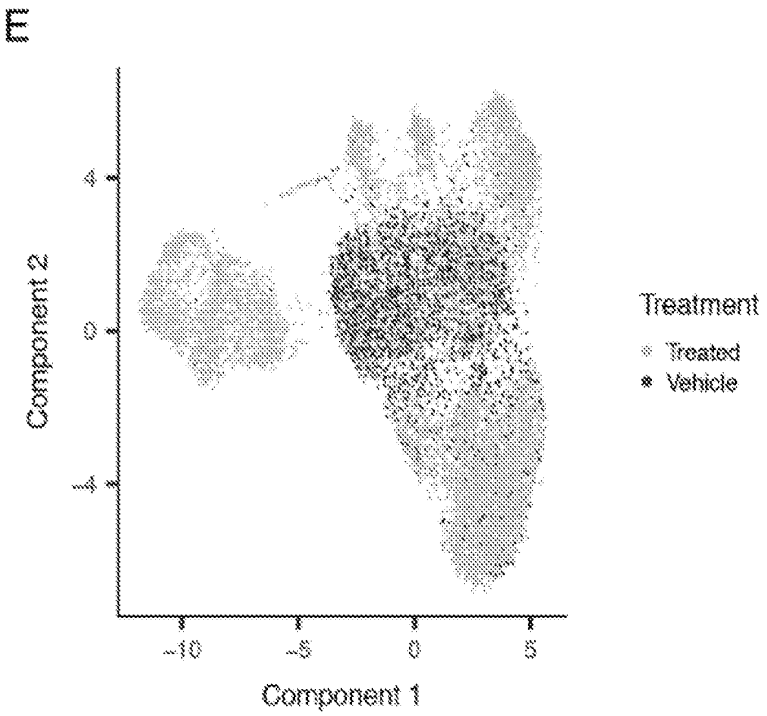

E
Component 2
4
0
−4
−10
−5
0
5
Component 1
Treatment
Treated
Vehicle

Fig. 7F

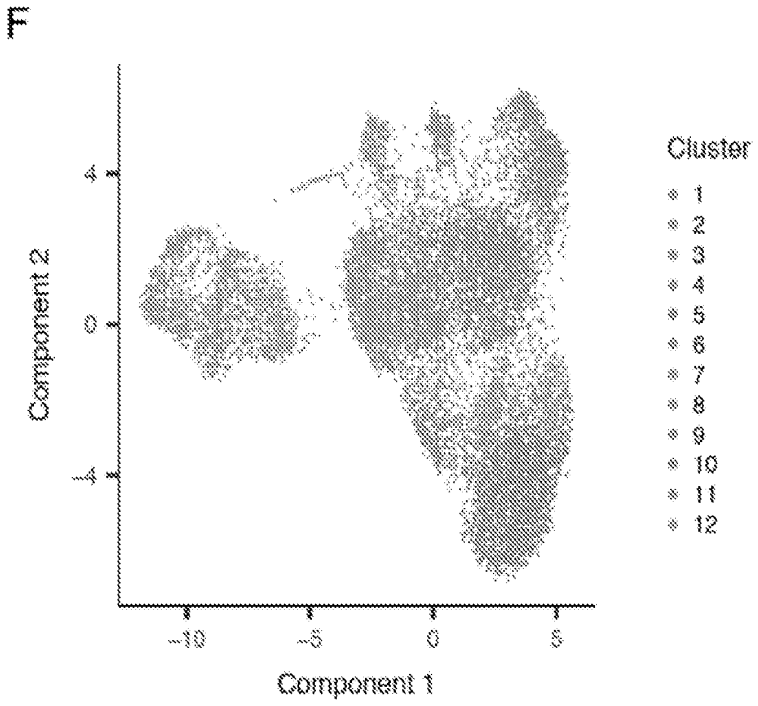

F
Component 2
4
0
−4
−10
−5
0
5
Component 1
Cluster
1
2
3
4
5
6
7
8
9
10
11
12

Fig. 7G

G

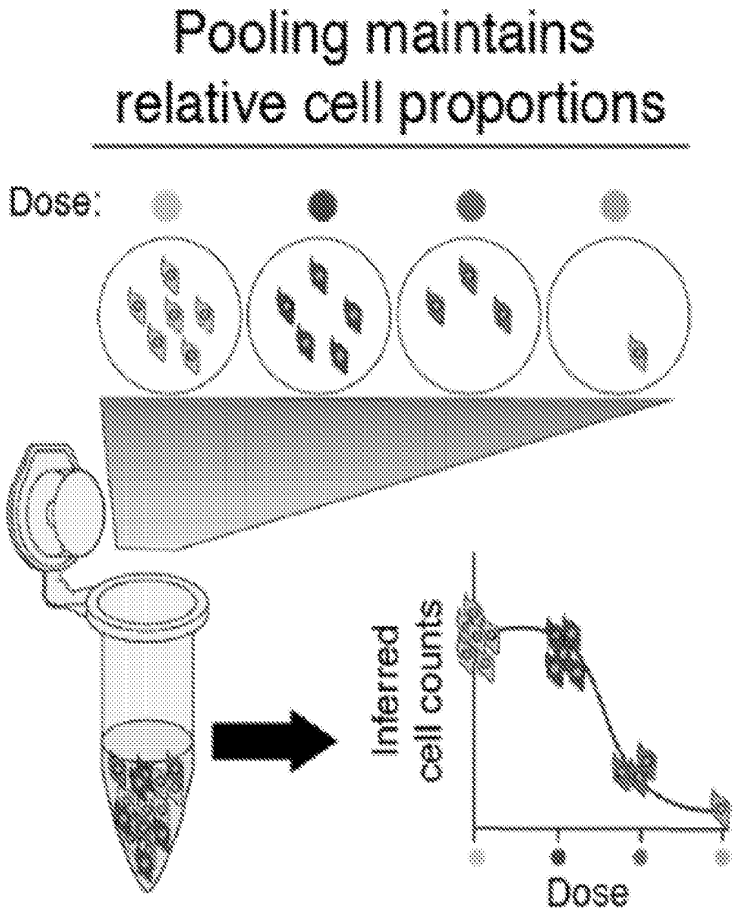

Pooling maintains
relative cell proportions
Dose:
Inferred
cell counts
Dose

Fig. 7H

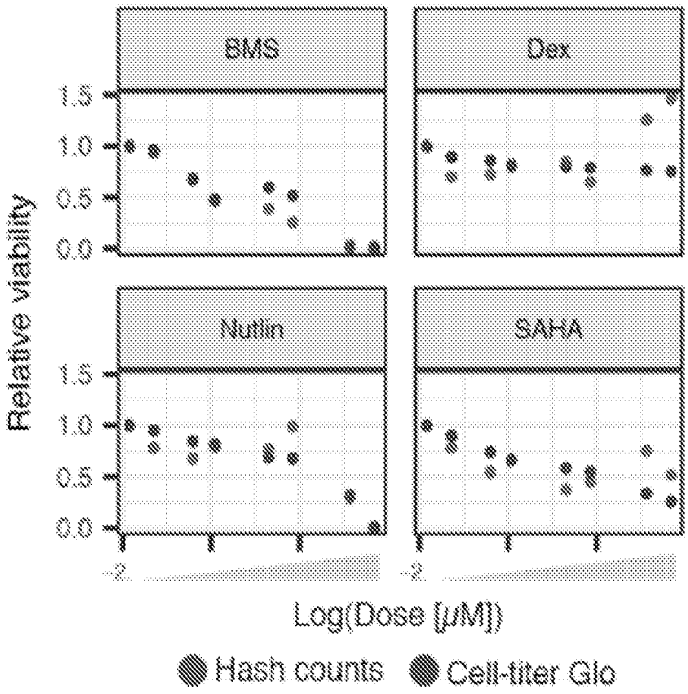
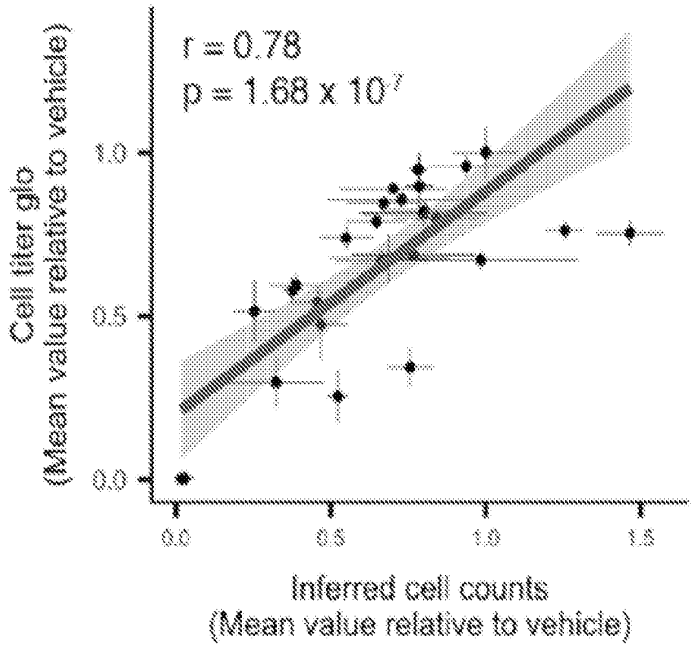

BMS
Dex
Nutlin
SAHA
Relative viability
1.5
1.0
0.5
0.0
-2
Log(Dose [μM])
Hash counts
Cell-titer Glo

Fig. 7I


r = 0.78
p = 1.68 x 10⁻⁷
Cell titer glo
(Mean value relative to vehicle)
1.0
0.5
0.0
0.0
0.5
1.0
1.5
Inferred cell counts
(Mean value relative to vehicle)

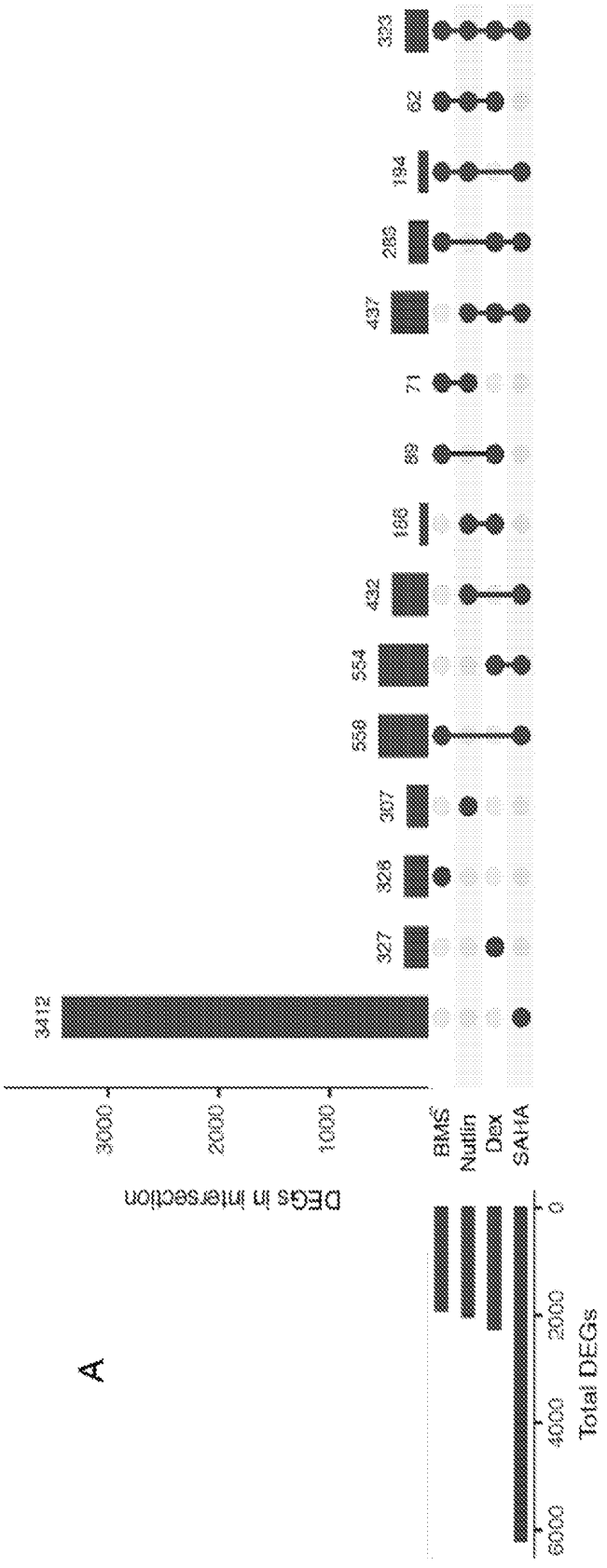

A
DEGs in intersection
3000
2000
1000
3412
327
328
307
538
554
432
166
89
71
437
283
194
62
323
BMS
Nutlin
Dex
SAHA
Total DEGs
0
2000
4000
6000

Fig. 8A

Fig. 8B

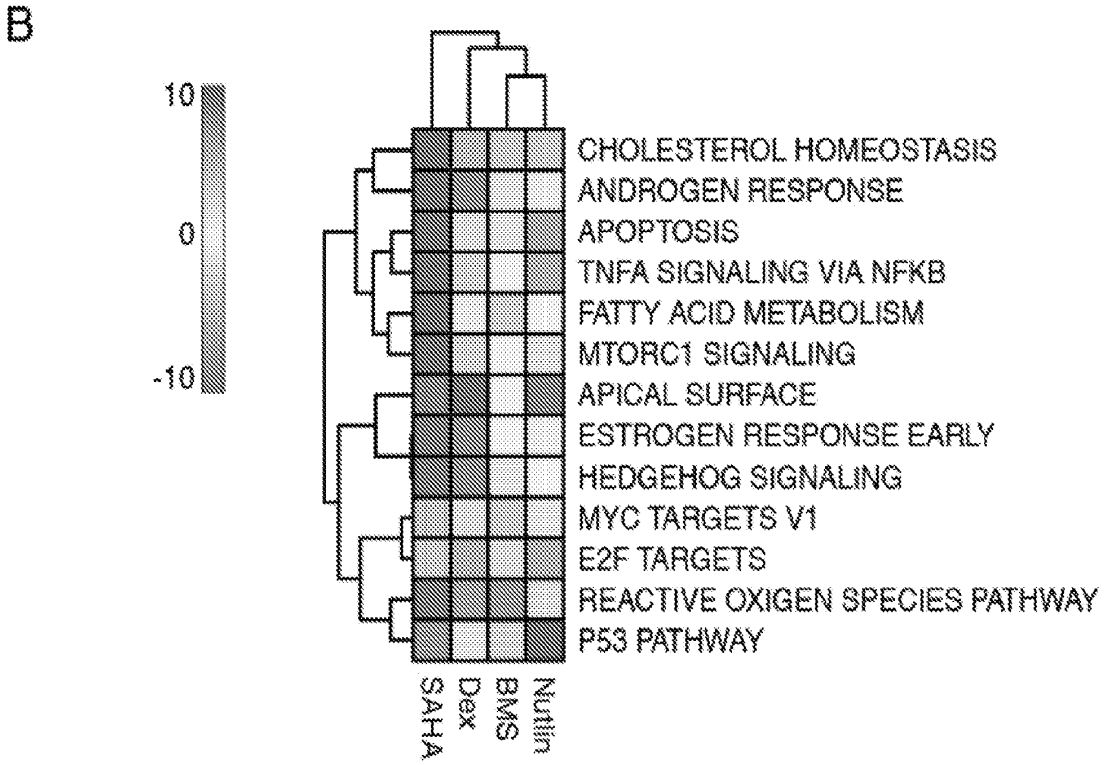

B
10
0
-10
CHOLESTEROL HOMEOSTASIS
ANDROGEN RESPONSE
APOPTOSIS
TNFA SIGNALING VIA NFKB
FATTY ACID METABOLISM
MTORC1 SIGNALING
APICAL SURFACE
ESTROGEN RESPONSE EARLY
HEDGEHOG SIGNALING
MYC TARGETS V1
E2F TARGETS
REACTIVE OXIGEN SPECIES PATHWAY
P53 PATHWAY
SAHA
Dex
BMS
Nutlin

Fig. 9A

A

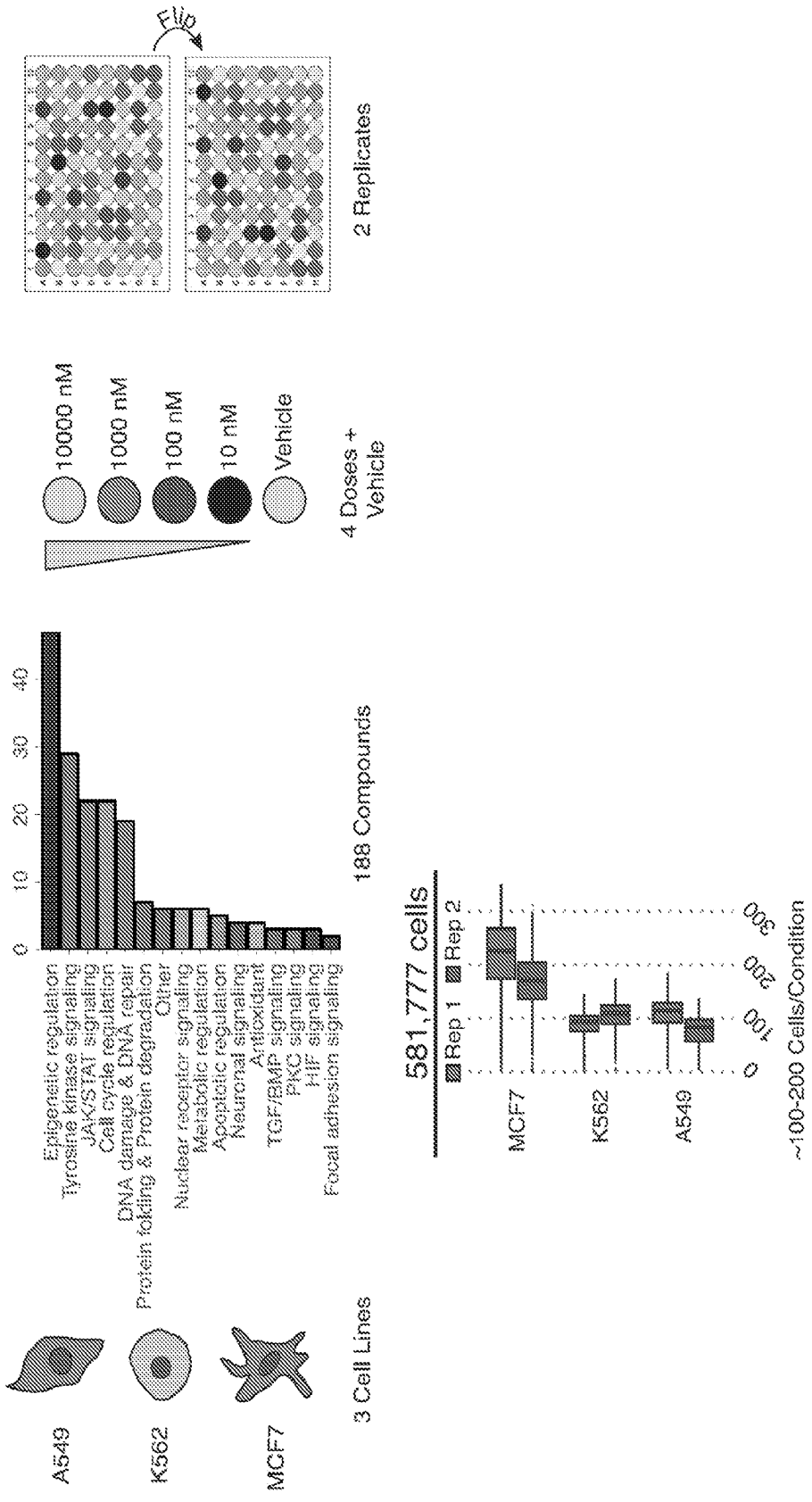
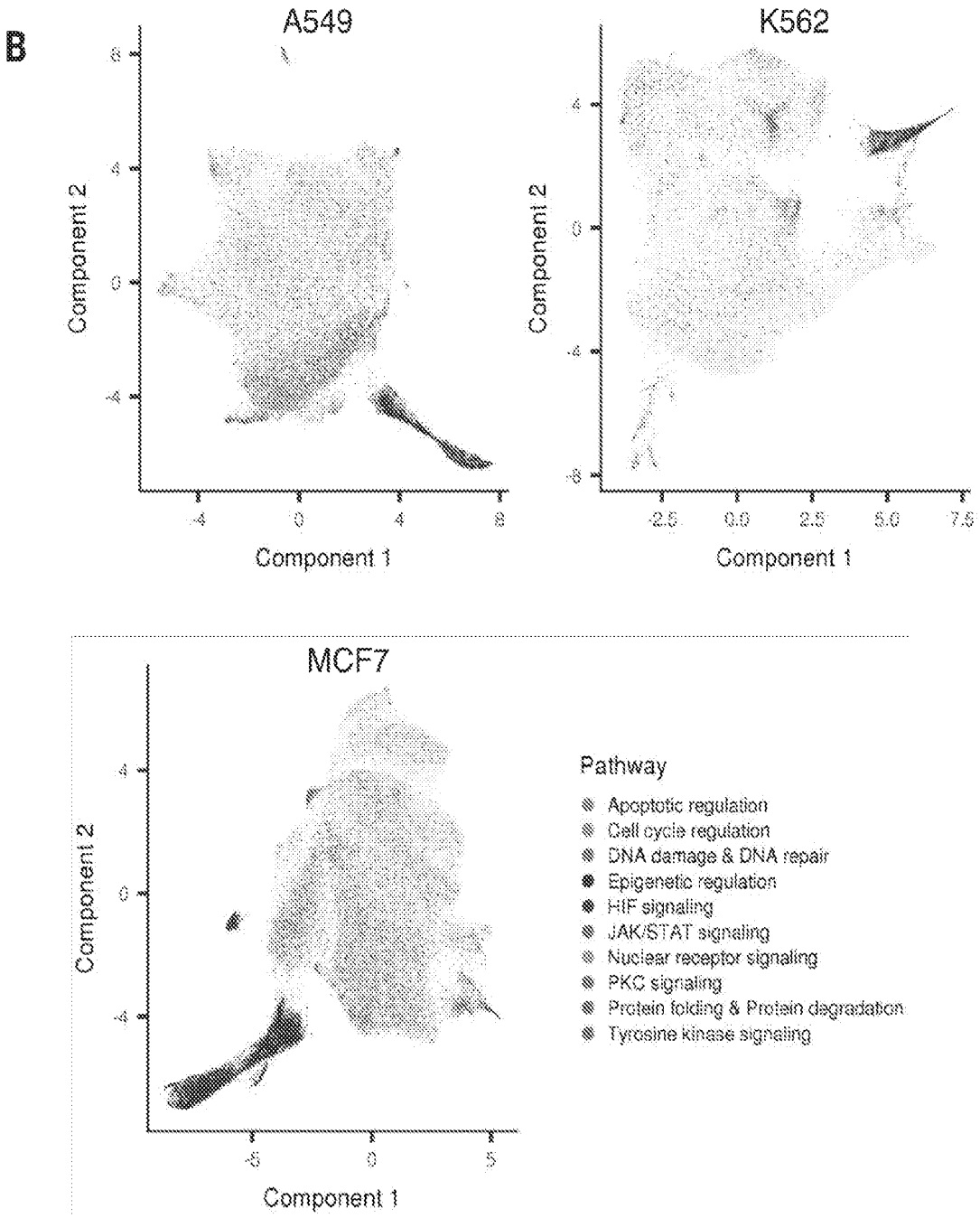

4,608 conditions - 24 hours post treatment
A549
K562
MCF7
3 Cell Lines
0
10
20
30
40
Epigenetic regulation
Tyrosine kinase signaling
JAK/STAT signaling
Cell cycle regulation
DNA damage & DNA repair
Protein folding & Protein degradation
Other
Nuclear receptor signaling
Metabolic regulation
Apoptotic regulation
Neuronal signaling
Antioxidant
TGF/BMP signaling
PKC signaling
HIF signaling
Focal adhesion signaling
188 Compounds
10000 nM
1000 nM
100 nM
10 nM
Vehicle
4 Doses + Vehicle
Fix
2 Replicates
581,777 cells
Rep 1
Rep 2
MCF7
K562
A549
0
100
200
300
~100-200 Cells/Condition

Fig. 9B


B
A549
K562
MCF7
Component 2
Component 1
Pathway
Apoptotic regulation
Cell cycle regulation
DNA damage & DNA repair
Epigenetic regulation
HIF signaling
JAK/STAT signaling
Nuclear receptor signaling
PKC signaling
Protein folding & Protein degradation
Tyrosine kinase signaling

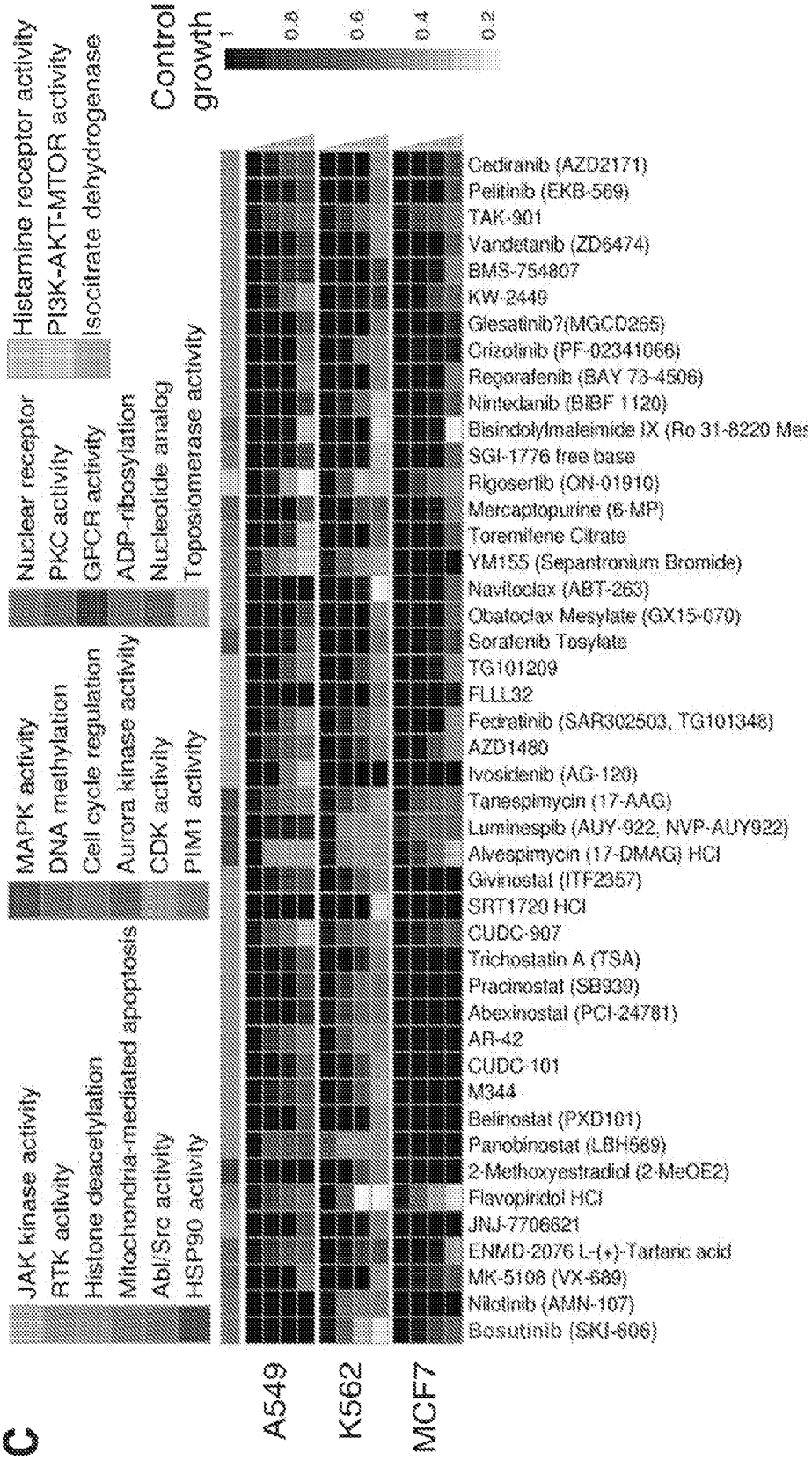

C
JAK kinase activity
RTK activity
Histone deacetylation
Mitochondria-mediated apoptosis
Abl/Src activity
HSP90 activity
MAPK activity
DNA methylation
Cell cycle regulation
Aurora kinase activity
CDK activity
PIM1 activity
Nuclear receptor
PKC activity
GPCR activity
ADP-ribosylation
Nucleotide analog
Topoisomerase activity
Histamine receptor activity
PI3K-AKT-MTOR activity
Isocitrate dehydrogenase
Control growth
1
0.8
0.6
0.4
0.2
Cediranib (AZD2171)
Peitinib (EKB-569)
TAK-901
Vandetanib (ZD6474)
BMS-754807
KW-2449
Glesatinib?(MGCD265)
Crizotinib (PF-02341066)
Regorafenib (BAY 73-4506)
Nintedanib (BIBF 1120)
Bisindolylmaleimide IX (Ro 31-8220 Mes
SGI-1776 free base
Rigosertib (ON-01910)
Mercaptopurine (6-MP)
Toremifene Citrate
YM155 (Sepantronium Bromide)
Navitoclax (ABT-263)
Obatoclax Mesylate (GX15-070)
Sorafenib Tosylate
TG101209
FLLL32
Fedratinib (SAR302503, TG101348)
AZD1480
Ivosidenib (AG-120)
Tanespimycin (17-AAG)
Luminespib (AUY-922, NVP-AUY922)
Alvespimycin (17-DMAG) HCl
Givinostat (ITF2357)
SRT1720 HCl
CUDC-907
Trichostatin A (TSA)
Pracinostat (SB939)
Abexinostat (PCI-24781)
AR-42
CUDC-101
M344
Belinostat (PXD101)
Panobinostat (LBH589)
2-Methoxyestradiol (2-MeOE2)
Flavopiridol HCl
JNJ-7706621
ENMD-2076 L-(+)-Tartaric acid
MK-5108 (VX-689)
Nilotinib (AMN-107)
Bosutinib (SKI-606)
A549
K562
MCF7

Fig. 9C

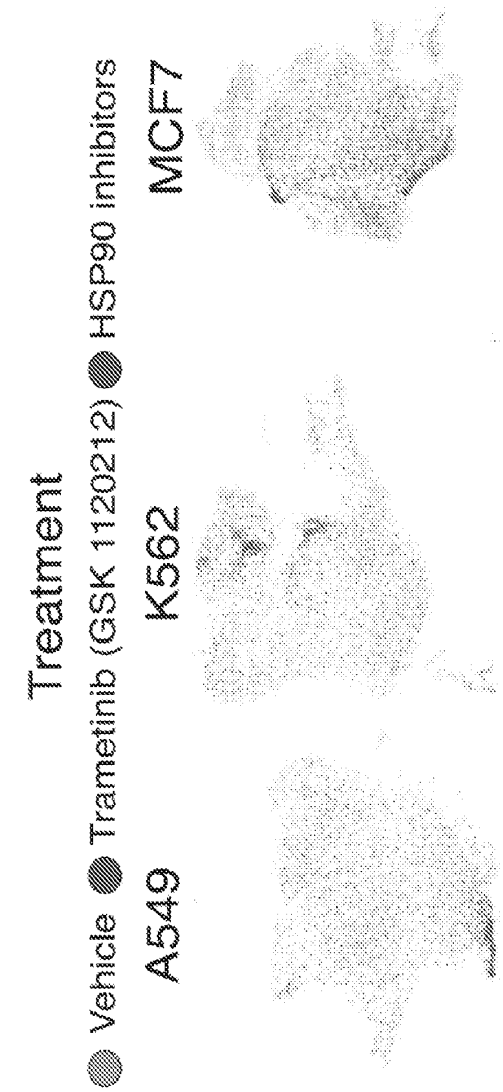

D
Treatment
Vehicle
Trametinib (GSK 1120212)
HSP90 inhibitors
A549
K562
MCF7

Fig. 9D

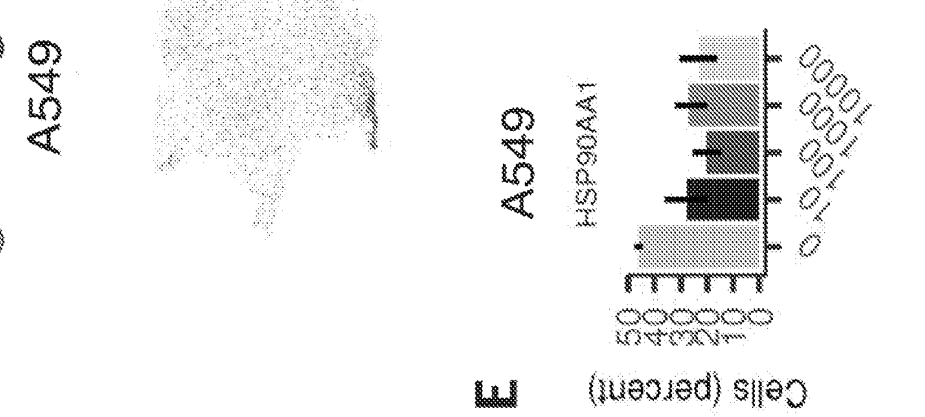

E
A549
K562
MCF7
HSP90AA1
Cells (percent)
[Trametinib (nM)]

Fig. 9E

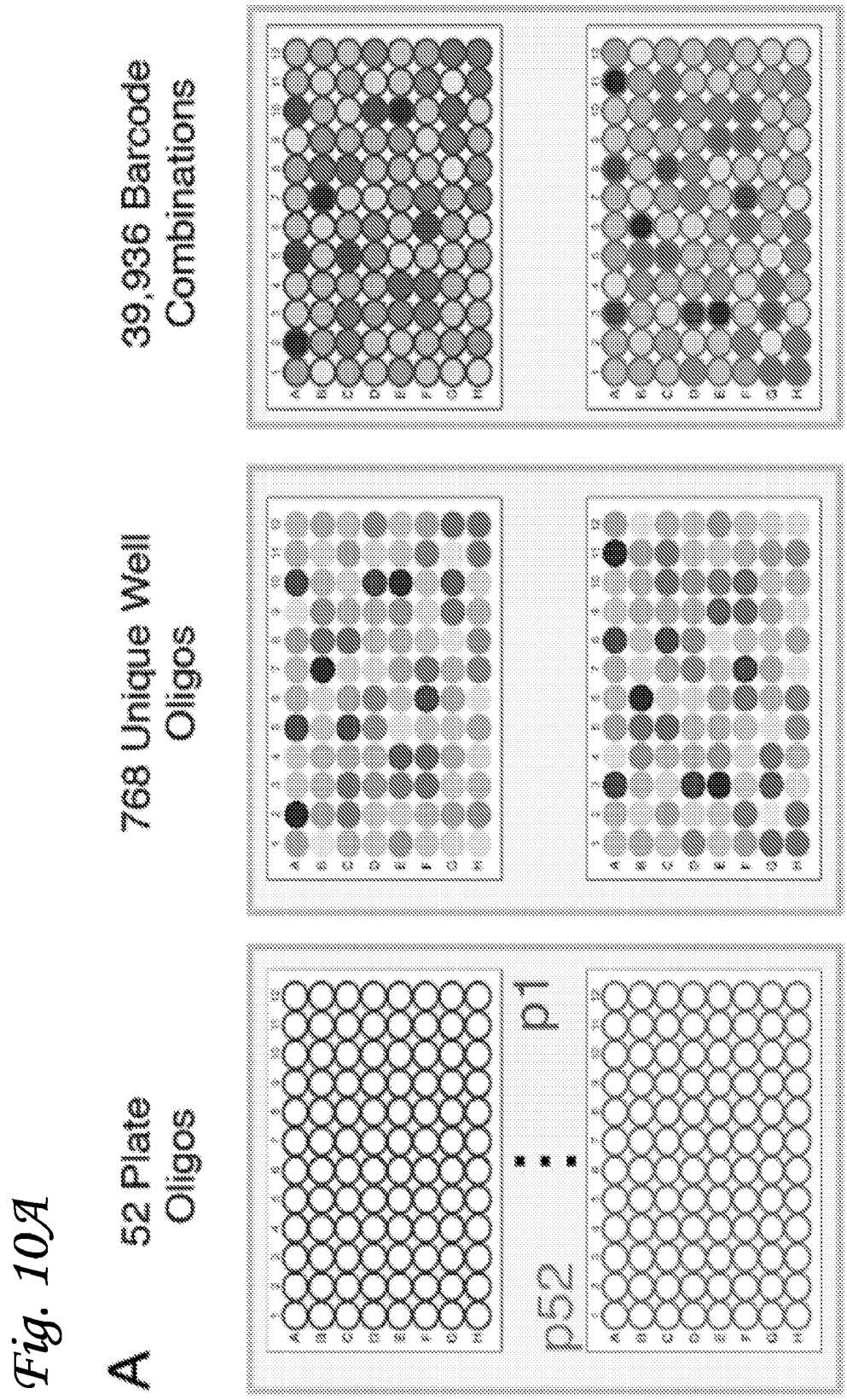

A
52 Plate Oligos
p1
p52
768 Unique Well Oligos
39,936 Barcode Combinations

Fig. 10A

Fig. 10B

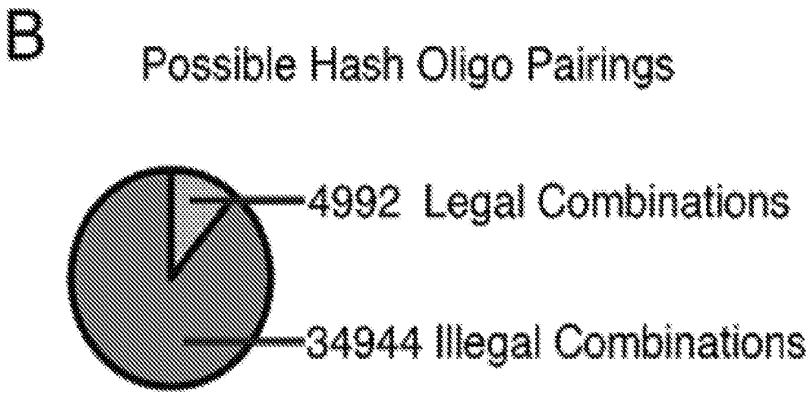


Fig. 10C

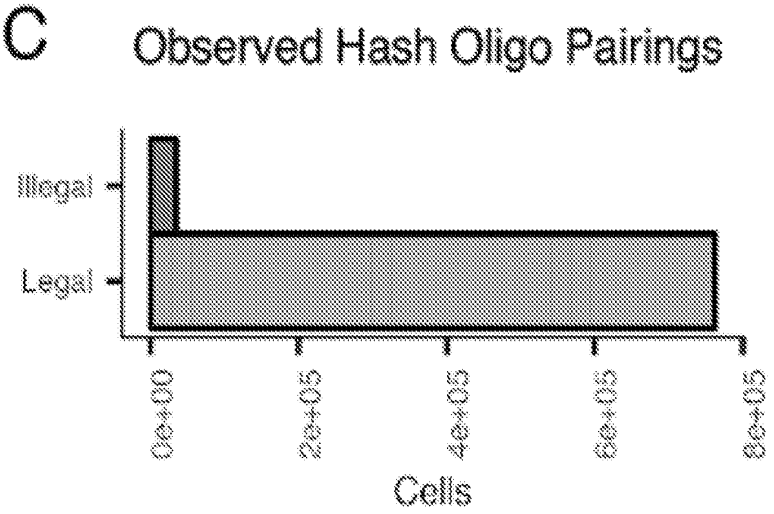


Fig. 10D

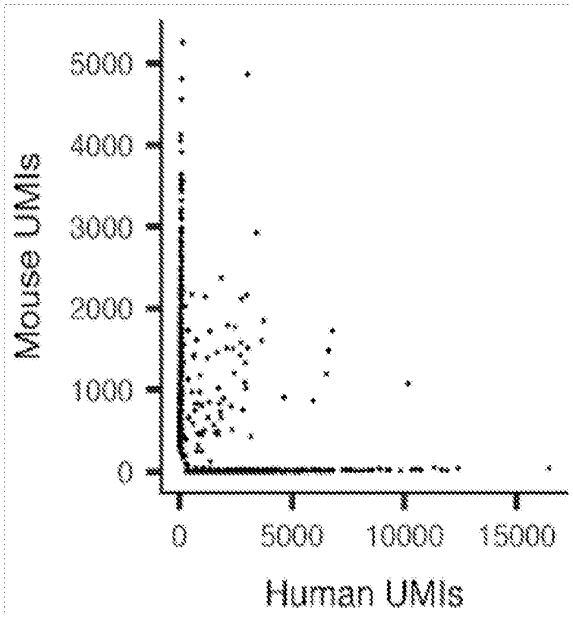


Fig. 10E

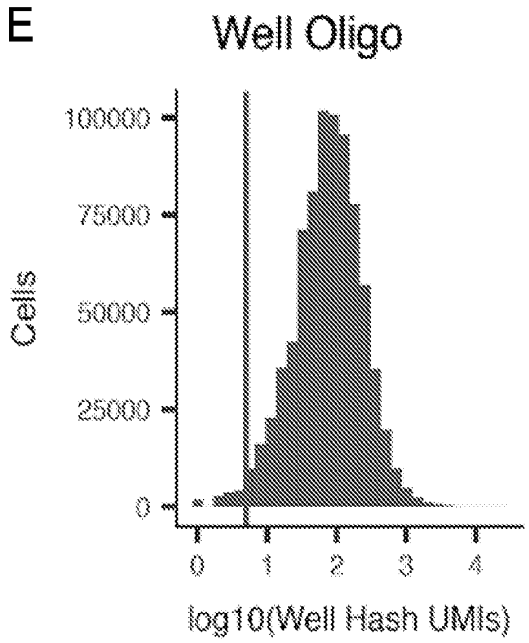


Fig. 10F

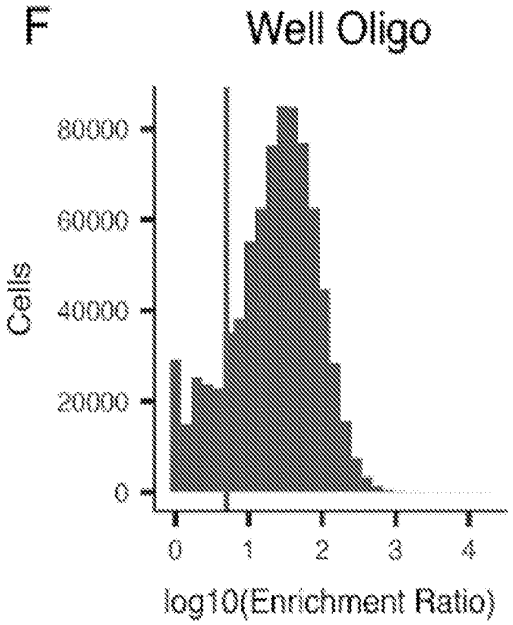


Fig. 10G

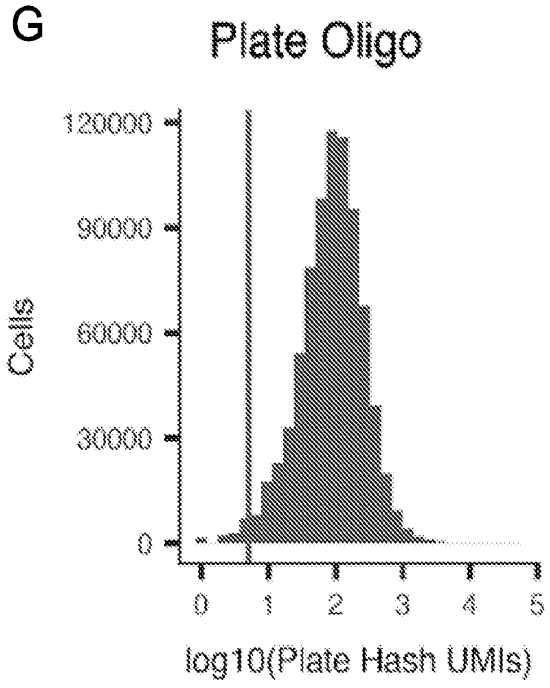
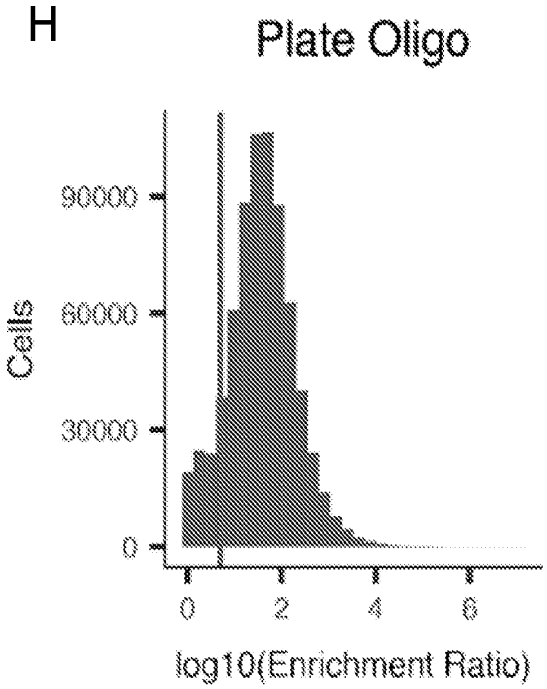


Fig. 10H



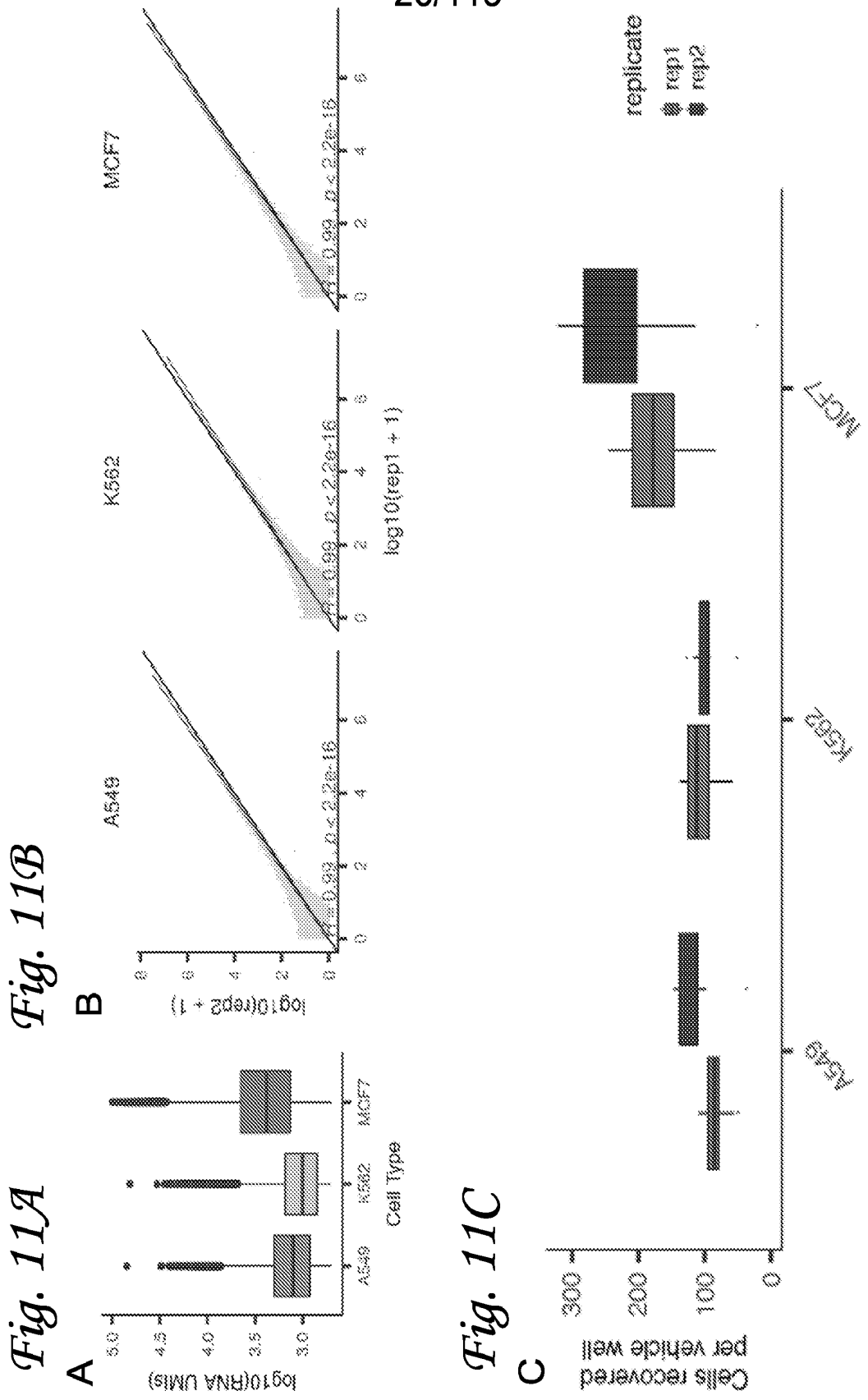


Fig. 11A

Fig. 11B

Fig. 11C

Fig. 12A

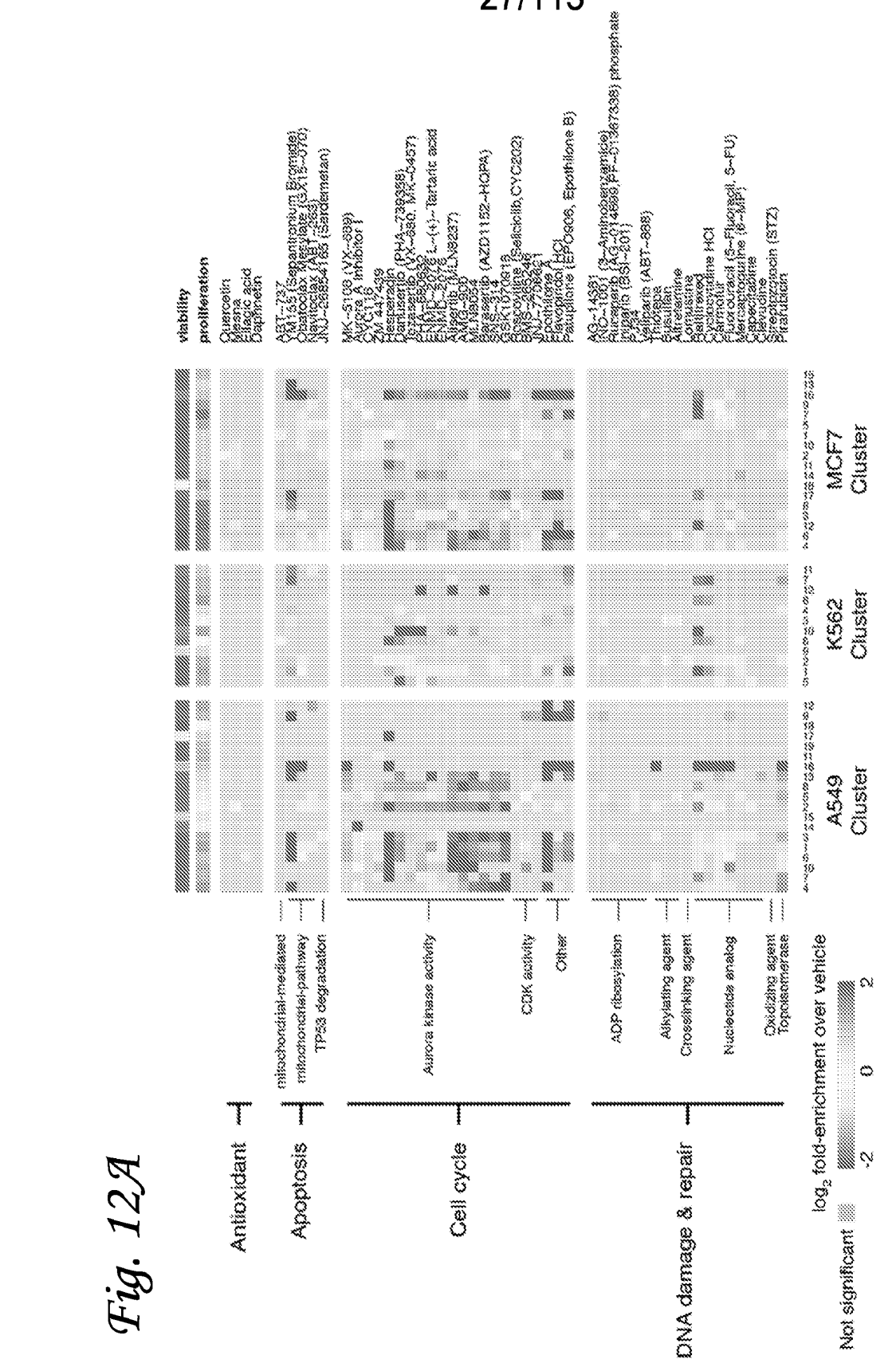

viability
proliferation
Quercetin
Mesna
Ellagic acid
Daphnetin
ABT-737
YM155 (Sepantronium Bromide)
Obatoclax Mesylate (GX15-070)
Navitoclax (ABT-263)
JNJ-26854165 (Serdemetan)
MK-51108 (VX-689)
Aurora A Inhibitor I
CYC116
ZM 447439
Hesperadin
Danusertib (PHA-739358)
Tozasertib (VX-680, MK-0457)
ENMD-2076 L-(+)- Tartaric acid
ENMD-2076
AMG-900
MLN8054
Alisertib (AZD1152-HQPA)
BMS-814
GSK1070916
Roscovitine (Seliciclib,CYC202)
BMS-265246
JNJ-7706621
Epothilone A
Flavopiridol HCl
Patupilone (EPOdoc6, Epothilone B)
AG-1433
NO-1001 (3-Aminobenzamide)
Rucaparib (AG-014699,PF-01367338) phosphate
Iniparib (BSI-201)
PJ34
Veliparib (ABT-888)
Thiotepa
Busulfan
Altretamine
Ifosfamide
Dacarbazine
Carmustine
Fluorouracil (5-Fluorouracil, 5-FU)
Mercaptopurine (6-MP)
Capecitabine
Cytarabine
Streptozocin (STZ)
Pralatrexate
Antioxidant
Apoptosis
Cell cycle
DNA damage & repair
mitochondrial-mediated
mitochondrial-pathway
TP53 degradation
Aurora kinase activity
CDK activity
Other
ADP ribosylation
Alkylating agent
Crosslinking agent
Nucleotide analog
Oxidizing agent
Topoisomerase
A549 Cluster
K562 Cluster
MCF7 Cluster
log2 fold-enrichment over vehicle
Not significant
-2
0
2

Fig. 12B

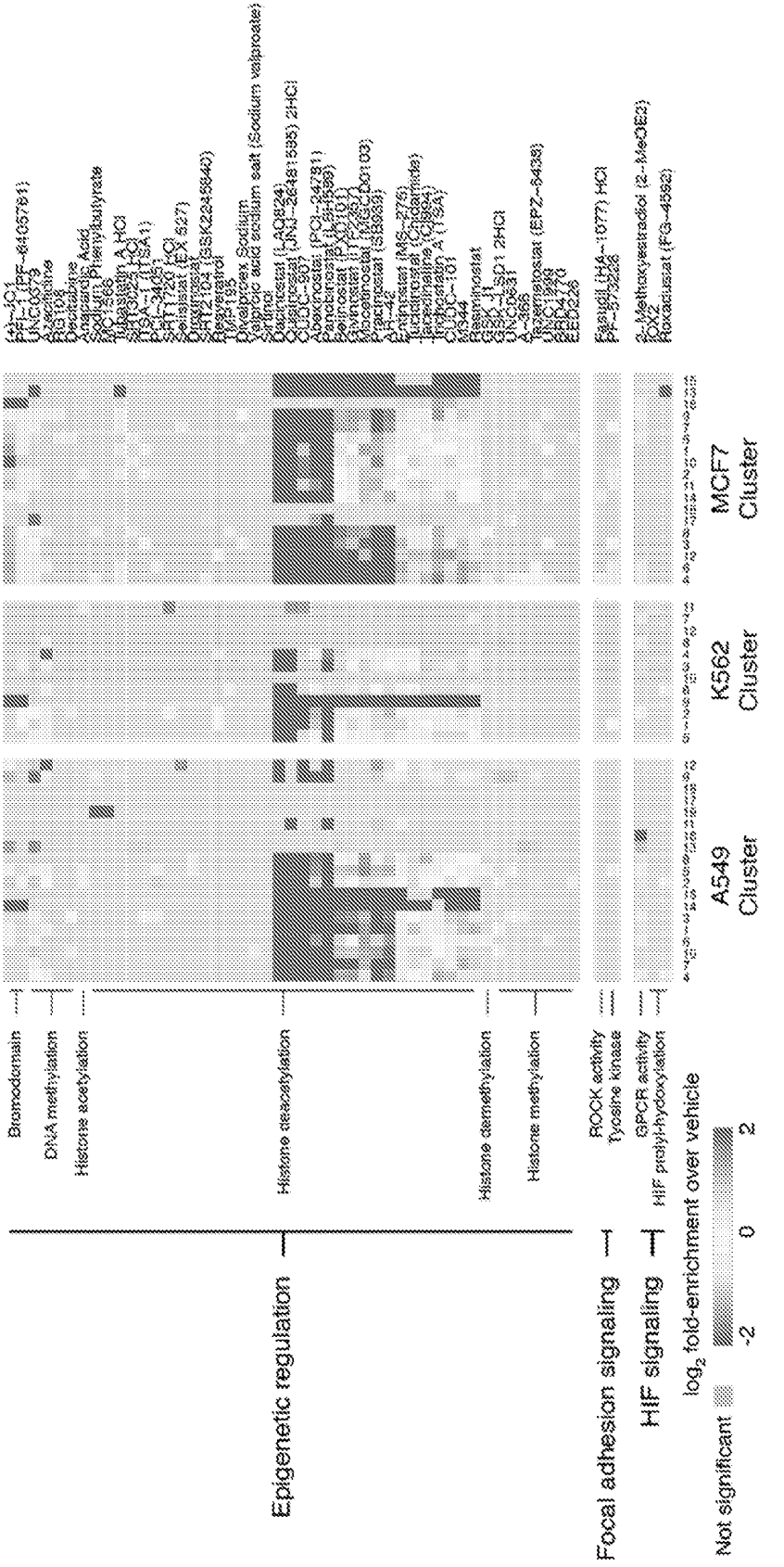

viability
proliferation
(+)-JQ1
PFI-1 (PF-6405761)
UNC0379
Azacitidine
RG108
Decitabine
Anacardic Acid
Sodium Phenylbutyrate
MC1568
Trichostatin A (TSA)
TSA (Trichostatin A)
BRD-3491
SRT1720 HCl
Selisistat (EX 527)
Diphenisat
CRT0124 (GSK2248840)
Resveratrol
TMP195
Divalproex Sodium
Valproic acid sodium salt (Sodium valproate)
Entinostat (LAQ824)
Givinostat (JNJ-26481585) 2HCl
CUDC-907
Quisinostat (JNJ-26481585) 2HCl
Abexinostat (PCI-24781)
Panobinostat (LBH589)
Belinostat (PXD101)
Givinostat (ITF2357)
Pracinostat (SB939)
AR-42
Entinostat (MS-275)
Tucidinostat (Chidamide)
Tacedinaline (CI994)
Trichostatin A (TSA)
CUDC-101
M344
Resminostat
GSK-J1
GSK-LSD1 2HCl
UNC0631
A-366
Tranylcypromine (EPZ-6438)
GSK343
UNC1999
EED226
Ezatiostat (TLK-19217) HCl
PP-372228
5-Methoxytryptophol (5-MTP)
ROX2
Roxadustat (FG-4592)
Bromodomain
DNA methylation
Histone acetylation
Histone deacetylation
Histone demethylation
Histone methylation
Epigenetic regulation
ROCK activity
Tyrosine kinase
Focal adhesion signaling
GPCR activity
HIF prolyl-hydroxylation
HIF signaling
A549 Cluster
K562 Cluster
MCF7 Cluster
log2 fold-enrichment over vehicle
Not significant
-2
0
2

Fig. 12C

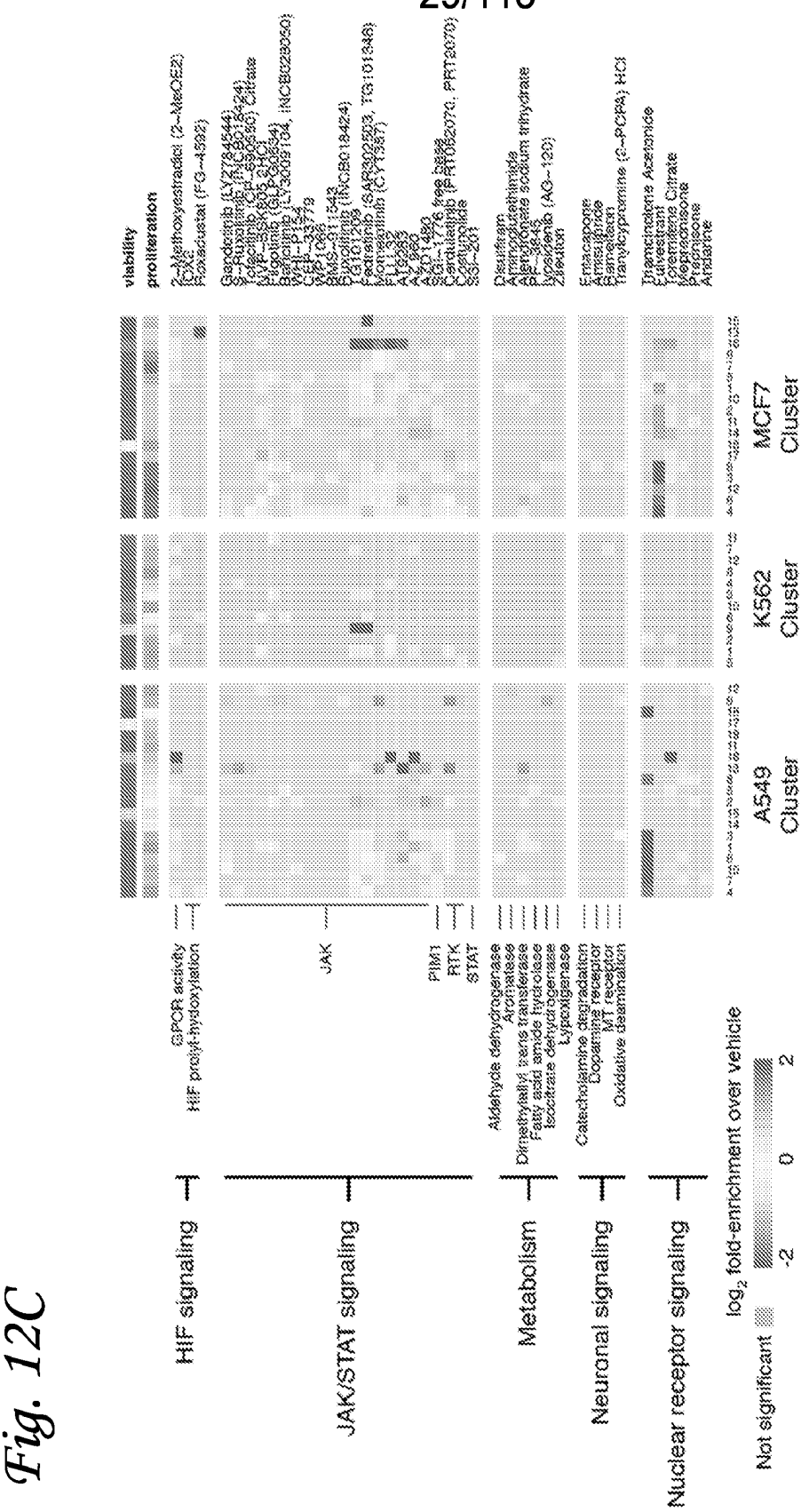

viability
proliferation
(2-Methoxyestradiol (2-MeOE2)
Roxadustat (FG-4592)
Gandotinib (LY2784544)
Ruxolitinib (INCB018424)
Fedratinib (SAR302503, TG101348)
Momelotinib (CYT387)
Pacritinib (SB1518)
AZD1480
AT9283
AG-014699
Crizotinib (PF-02341066, PF02341066)
Ceritinib (LDK378)
SGI-1776 free base
Disulfiram
Ammonium tetrathiomolybdate
Aminoglutethimide
Aminoglutethimide sodium trihydrate
BAY 36-5448
Lycorine
Lycorine hydrochloride (AG-120)
Ziprasidone
Entacapone
Amsilarine
Gamolenic acid
Tranylcypromine (2-PCPA) HCl
Diamcinolone Acetonide
Fulvestrant
Toremifene Citrate
Meprednisone
Prednisone
Andarine
HIF signaling
JAK/STAT signaling
Metabolism
Neuronal signaling
Nuclear receptor signaling
GPCR activity
HIF prolyl-hydroxylation
JAK
PIM1
RTK
STAT
Aldehyde dehydrogenase
Aromatase
Dimethylallyl trans transferase
Fatty acid amide hydrolase
Isocitrate dehydrogenase
Lipoxygenase
Catecholamine degradation
Dopamine receptor
MT receptor
Oxidative deamination
A549 Cluster
K562 Cluster
MCF7 Cluster
log2 fold-enrichment over vehicle
Not significant
-2
0
2

Fig. 12D

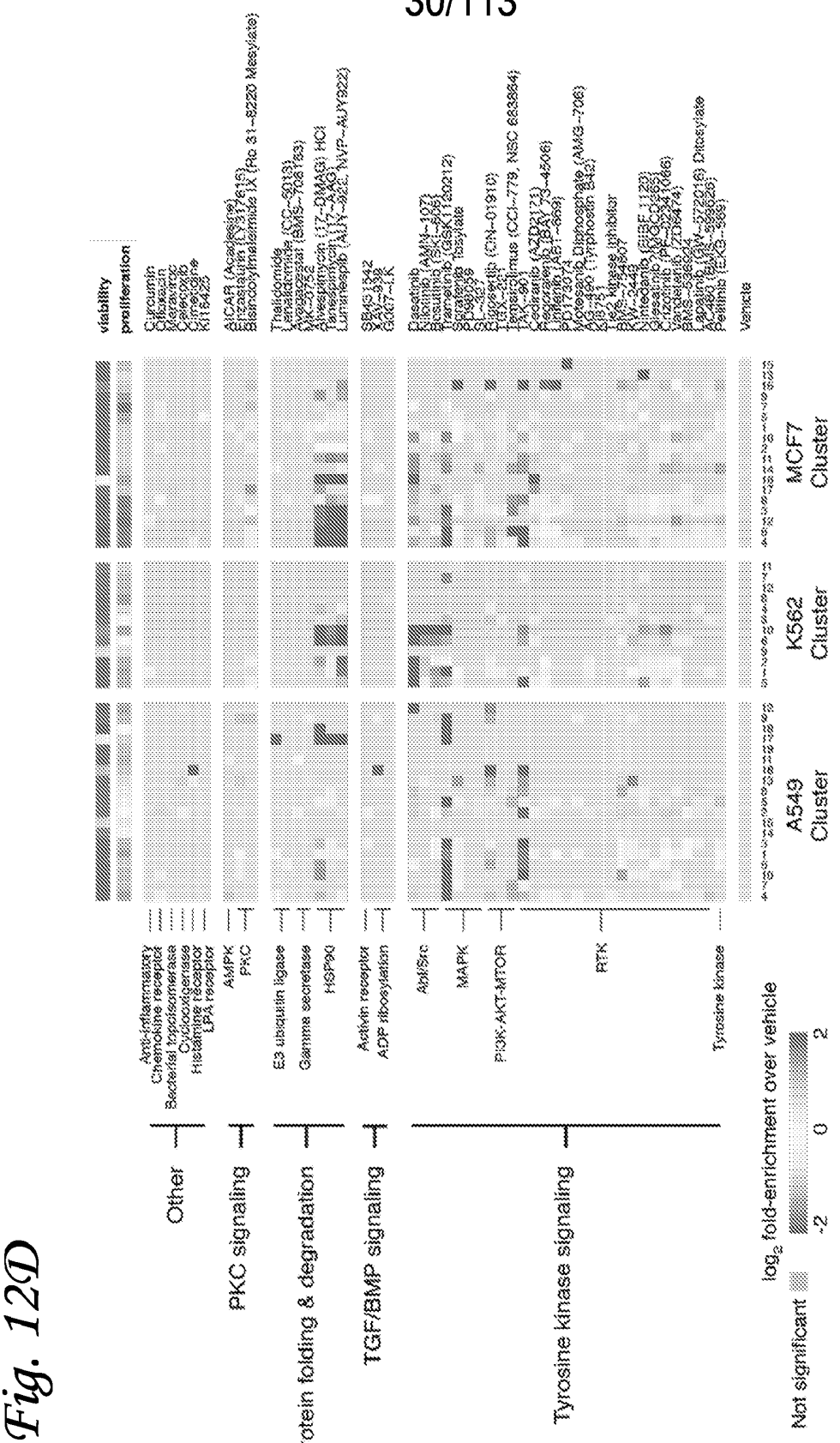

viability
proliferation
Curcumin
Oligomycin
Ceramide
Cinnarizine
KIN1542
AICAR (Acadesine)
Enzastaurin (LY317615)
Bisindolylmaleimide IX (Ro 31-8220 Mesylate)
Thalidomide
Lenalidomide (CC-5013)
MK-28752
Alvespimycin (17-DMAG) HCl
Tanespimycin (17-AAG)
Luminespib (AUY-922, NVP-AUY922)
SB431542
A83-01
GW627-1K
Dasatinib
Nilotinib (AMN-107)
Roscovitine (Seliciclib, CYC202)
Triciribine (API-2, NSC 154020)
SP600125
SI-2
Bosutinib (SKI-606)
TAK-901
Temsirolimus (CCI-779, NSC 683864)
PAC-1
Cediranib (AZD2171)
Rapamycin (AY22989)
Sunitinib (SU11248)
KD025 (SLx-2119)
AG-490 (Tyrphostin B42)
GSK461364
TG101348
JAK2 kinase inhibitor
BMS-754807
XMD8-92
Linifanib (ABT-869)
Gefitinib (IRESSA)
Crizotinib (PF-02341066)
Canertinib (CI-1033)
Pelitinib (EKB-569)
AZD6482 (KIN-193)
AZD8055
Vehicle
Other
Anti-inflammatory
Chemokine receptor
Bacterial lipopolysaccharide
Cyclooxygenase
Histamine receptor
LPA receptor
PKC signaling
AMPK
PKC
Protein folding & degradation
E3 ubiquitin ligase
Gamma secretase
HSP90
TGF/BMP signaling
Activin receptor
ADP ribosylation
Tyrosine kinase signaling
Abl/Src
MAPK
PI3K-AKT-MTOR
RTK
Tyrosine kinase
A549 Cluster
K562 Cluster
MCF7 Cluster
log2 fold-enrichment over vehicle
-2
0
2
Not significant

Fig. 13A

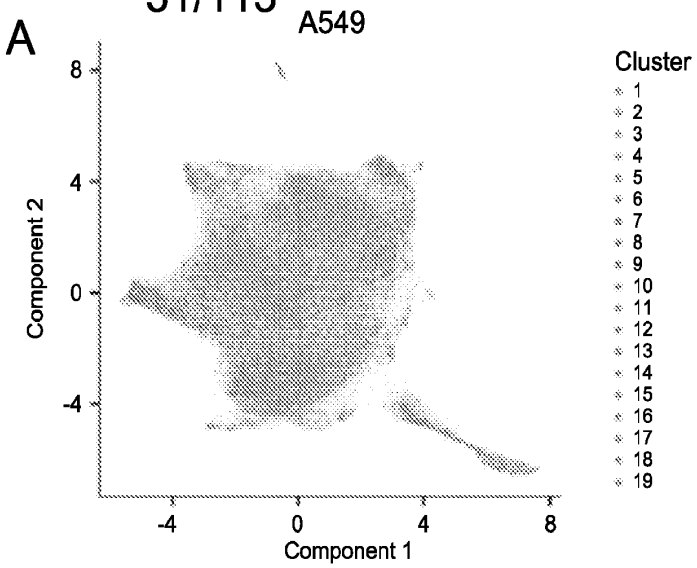


Fig. 13B

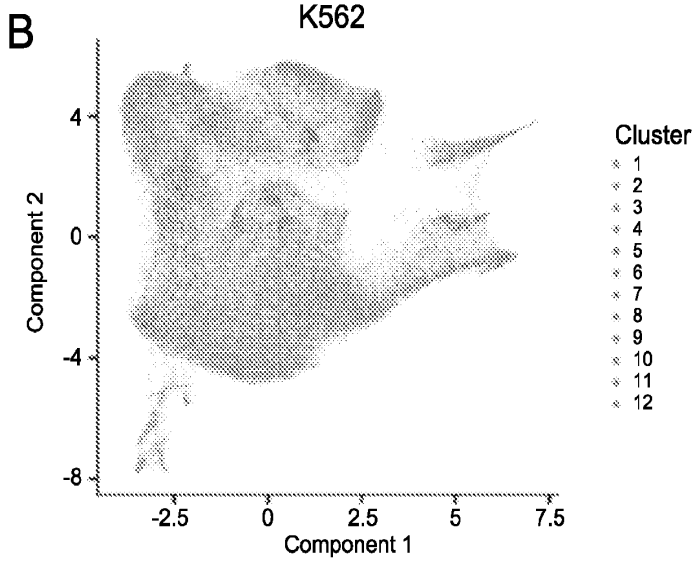


Fig. 13C

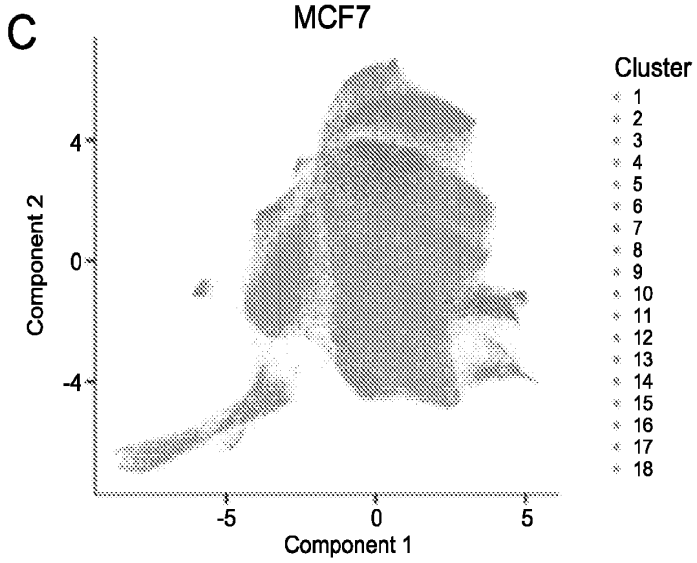


Fig. 13D

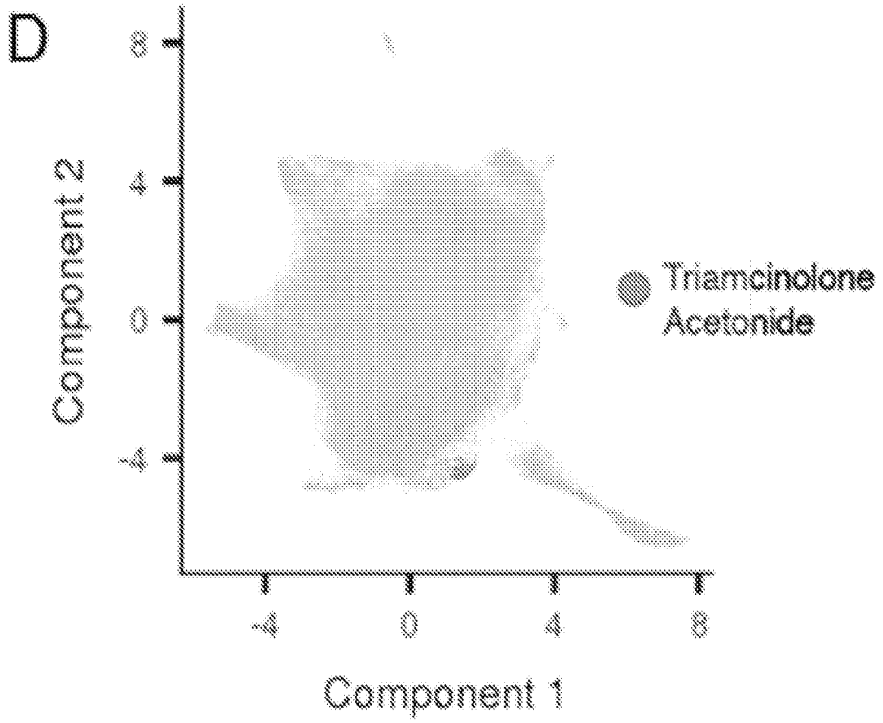


Fig. 13E

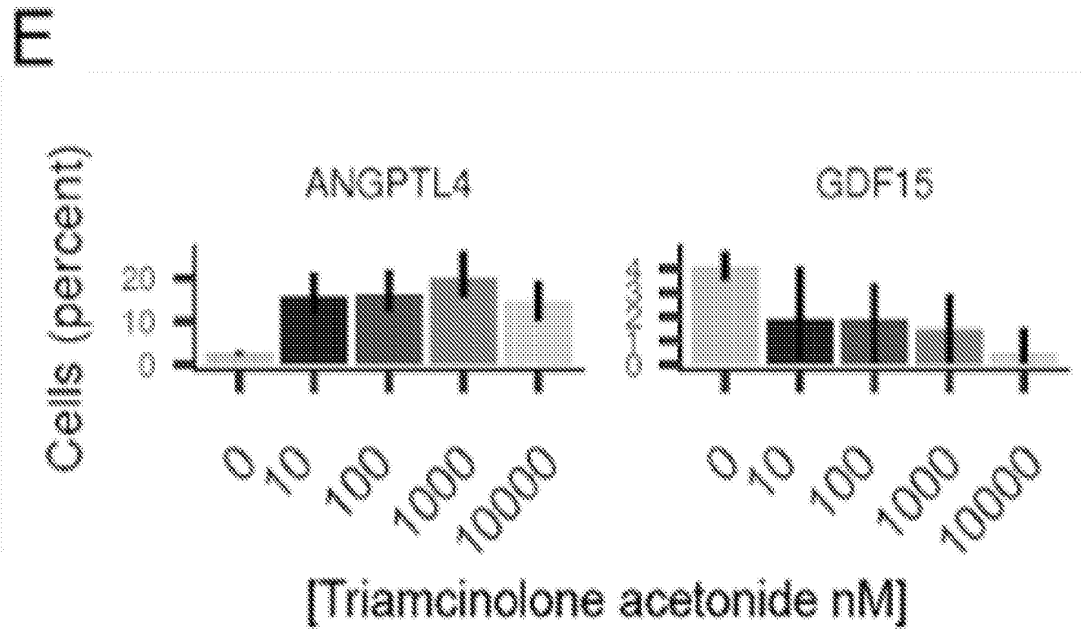


Fig. 13F

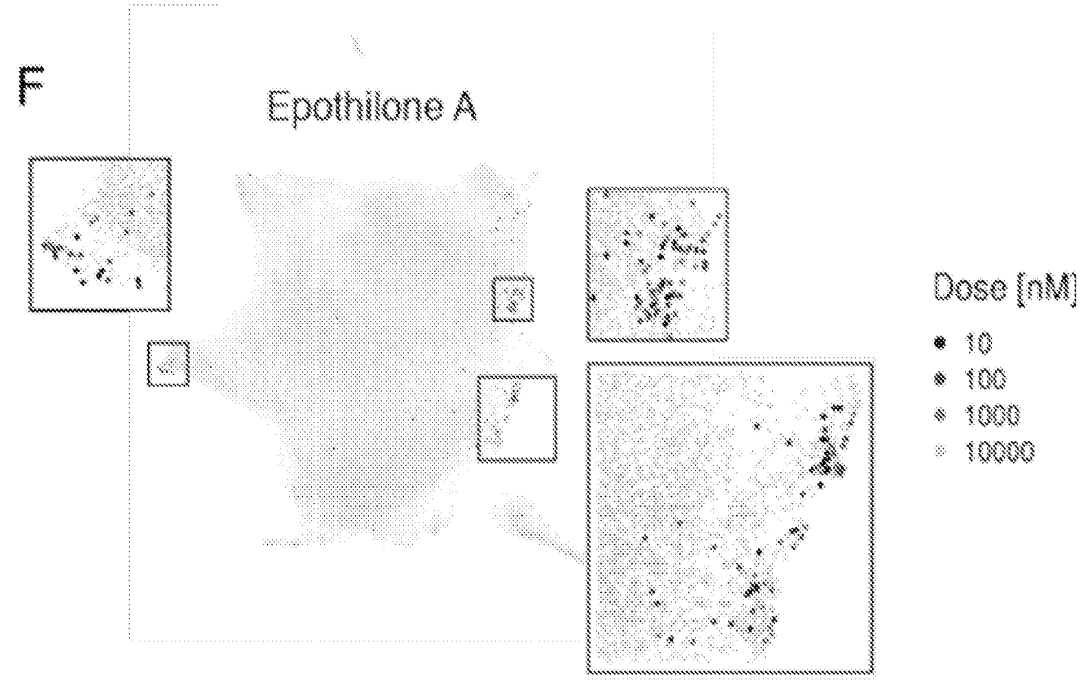
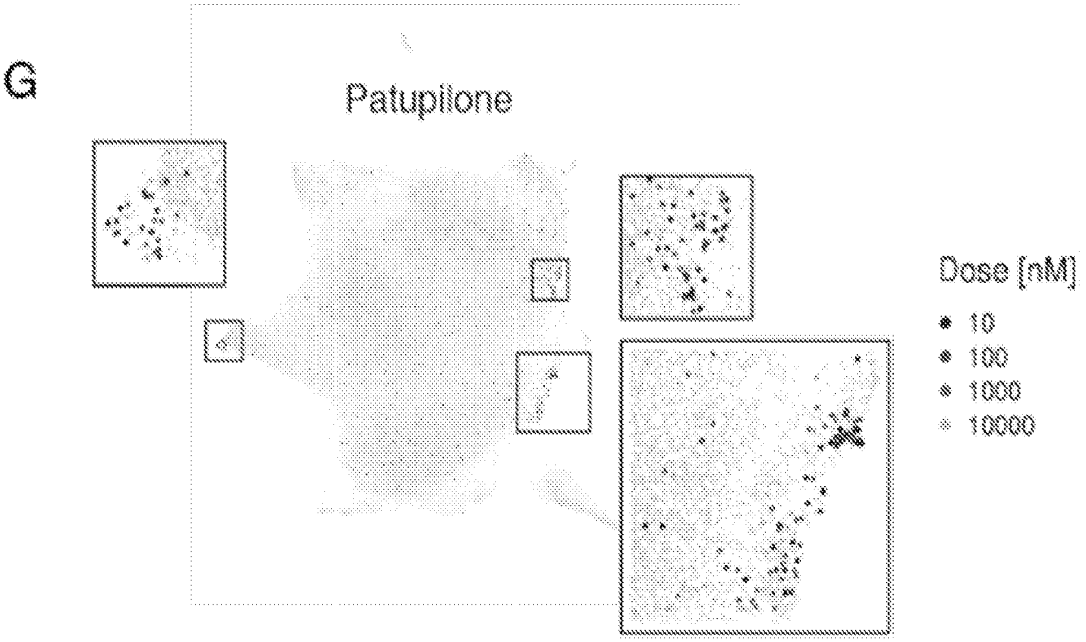


Fig. 13G



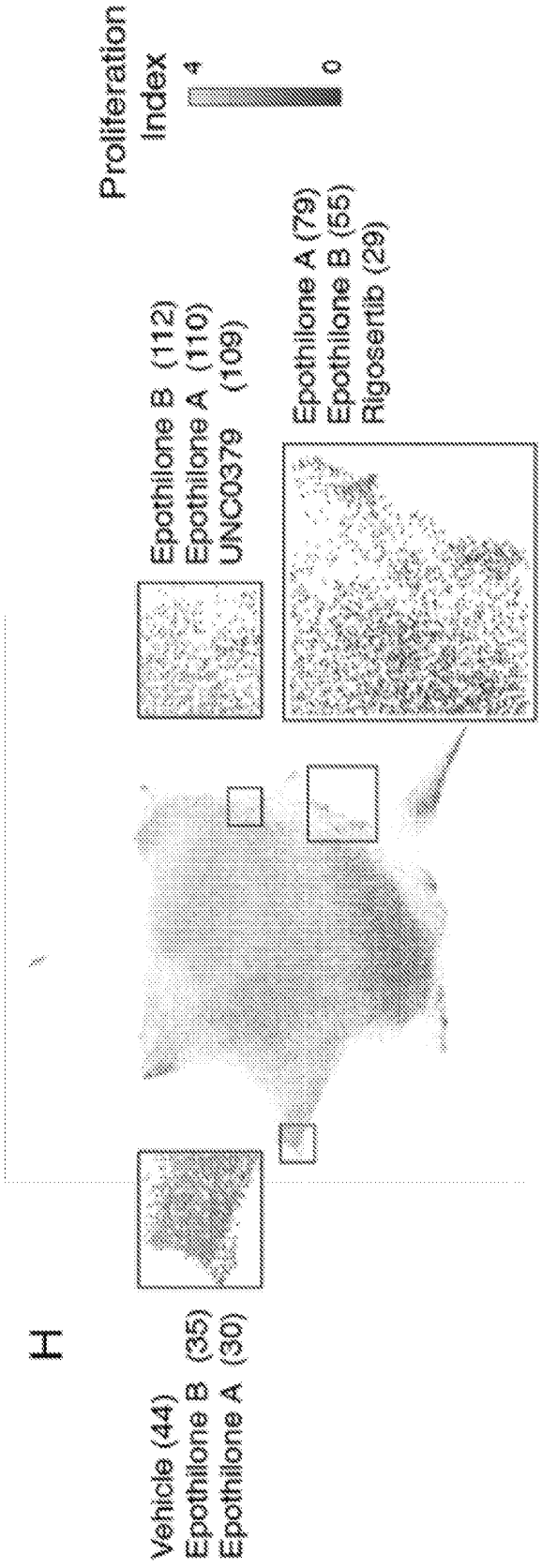

H
Vehicle (44)
Epothilone B (35)
Epothilone A (30)
Epothilone B (112)
Epothilone A (110)
UNC0379 (109)
Epothilone A (79)
Epothilone B (55)
Rigosertib (29)
Proliferation Index
4
0

Fig. 13H

Fig. 14

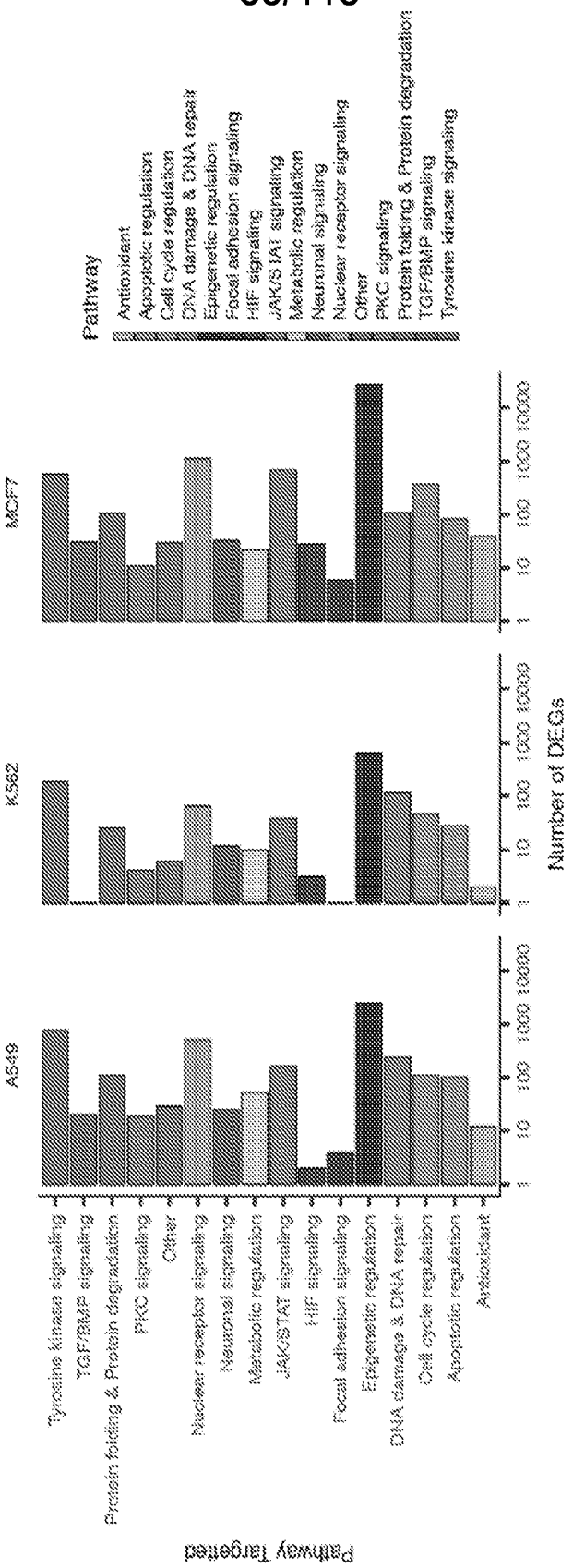

A549
K562
MCF7
Pathway
Antioxidant
Apoptotic regulation
Cell cycle regulation
DNA damage & DNA repair
Epigenetic regulation
Focal adhesion signaling
HIF signaling
JAK/STAT signaling
Metabolic regulation
Neuronal signaling
Nuclear receptor signaling
Other
PKC signaling
Protein folding & Protein degradation
TGF/BMP signaling
Tyrosine kinase signaling
Tyrosine kinase signaling
TGF/BMP signaling
Protein folding & Protein degradation
PKC signaling
Other
Nuclear receptor signaling
Neuronal signaling
Metabolic regulation
JAK/STAT signaling
HIF signaling
Focal adhesion signaling
Epigenetic regulation
DNA damage & DNA repair
Cell cycle regulation
Apoptotic regulation
Antioxidant
1
10
100
1000
10000
Number of DEGs
Pathway Targeted

Fig. 15A

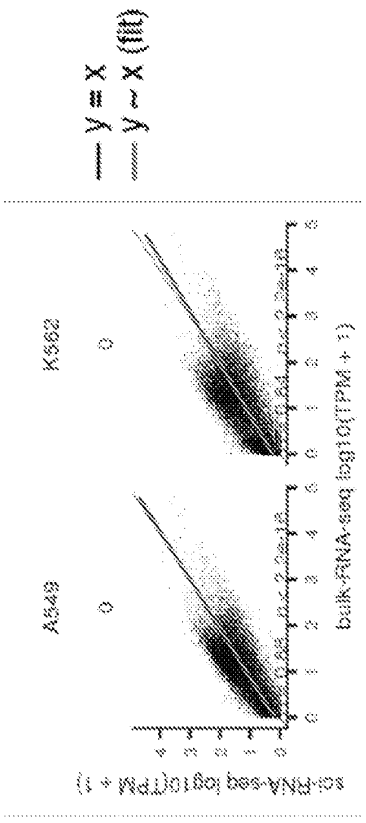


Fig. 15B

Fig. 16A

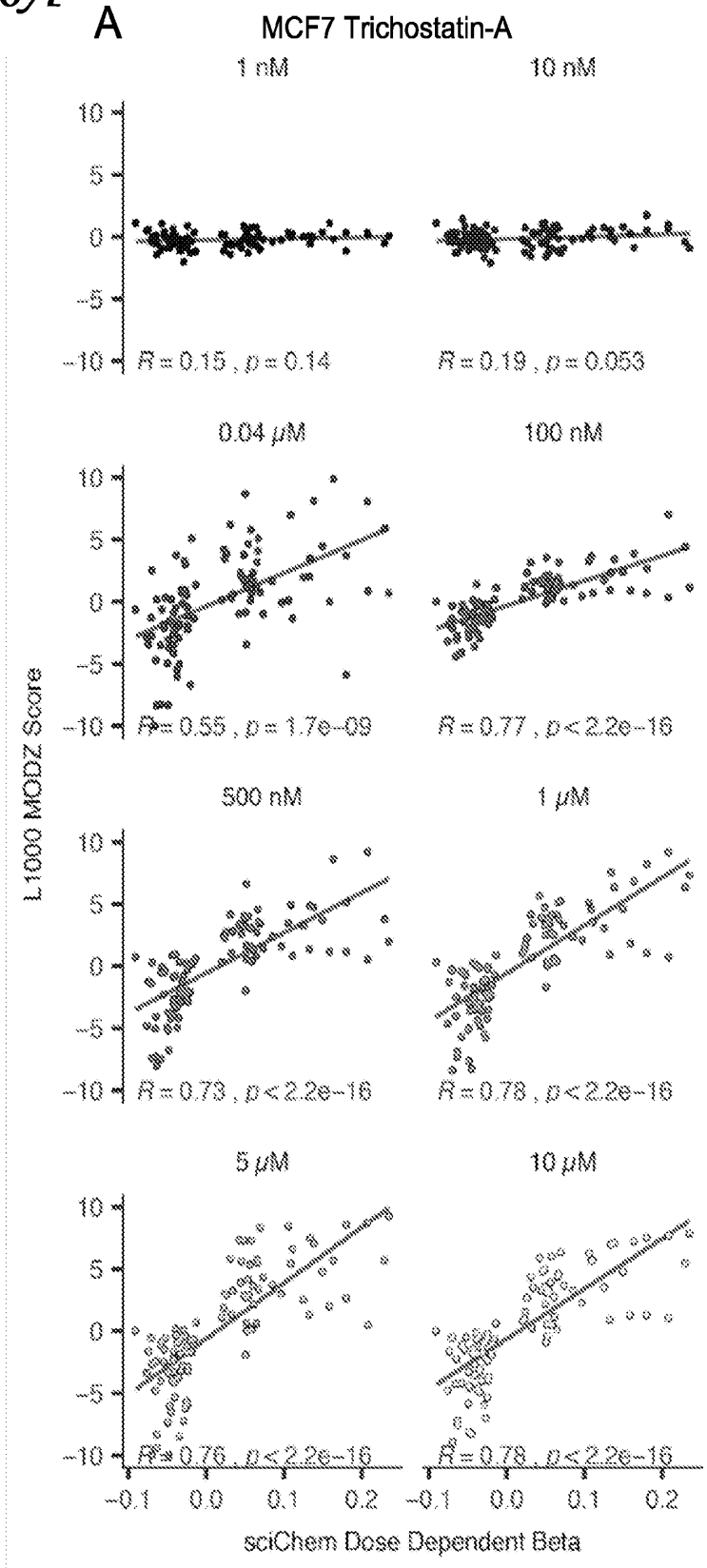

A
MCF7 Trichostatin-A
1 nM
10 nM
0.04 μM
100 nM
500 nM
1 μM
5 μM
10 μM
R = 0.15 , p = 0.14
R = 0.19 , p = 0.053
R = 0.55 , p = 1.7e−09
R = 0.77 , p < 2.2e−16
R = 0.73 , p < 2.2e−16
R = 0.78 , p < 2.2e−16
R = 0.76 , p < 2.2e−16
R = 0.78 , p < 2.2e−16
L1000 MODZ Score
sciChem Dose Dependent Beta

Fig. 16B

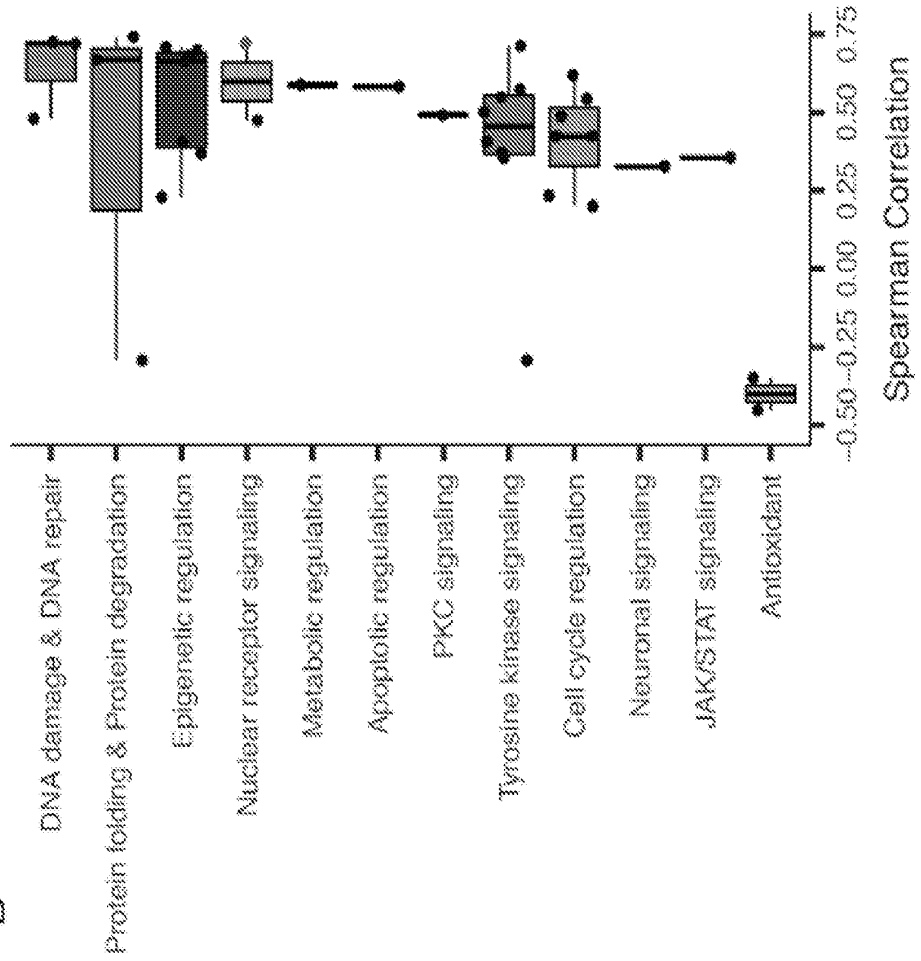
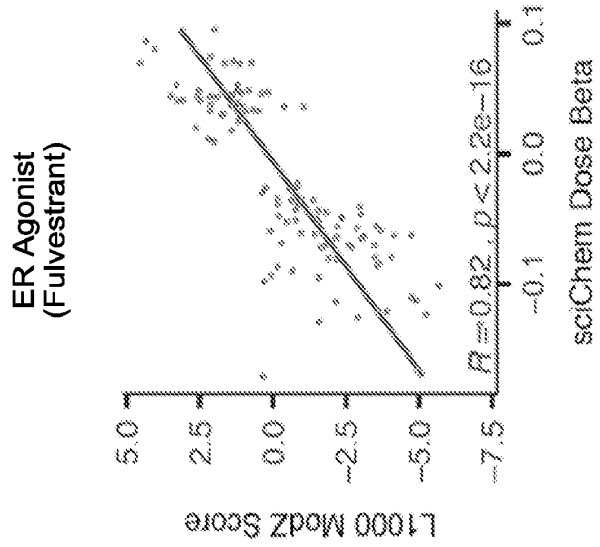


Fig. 16C



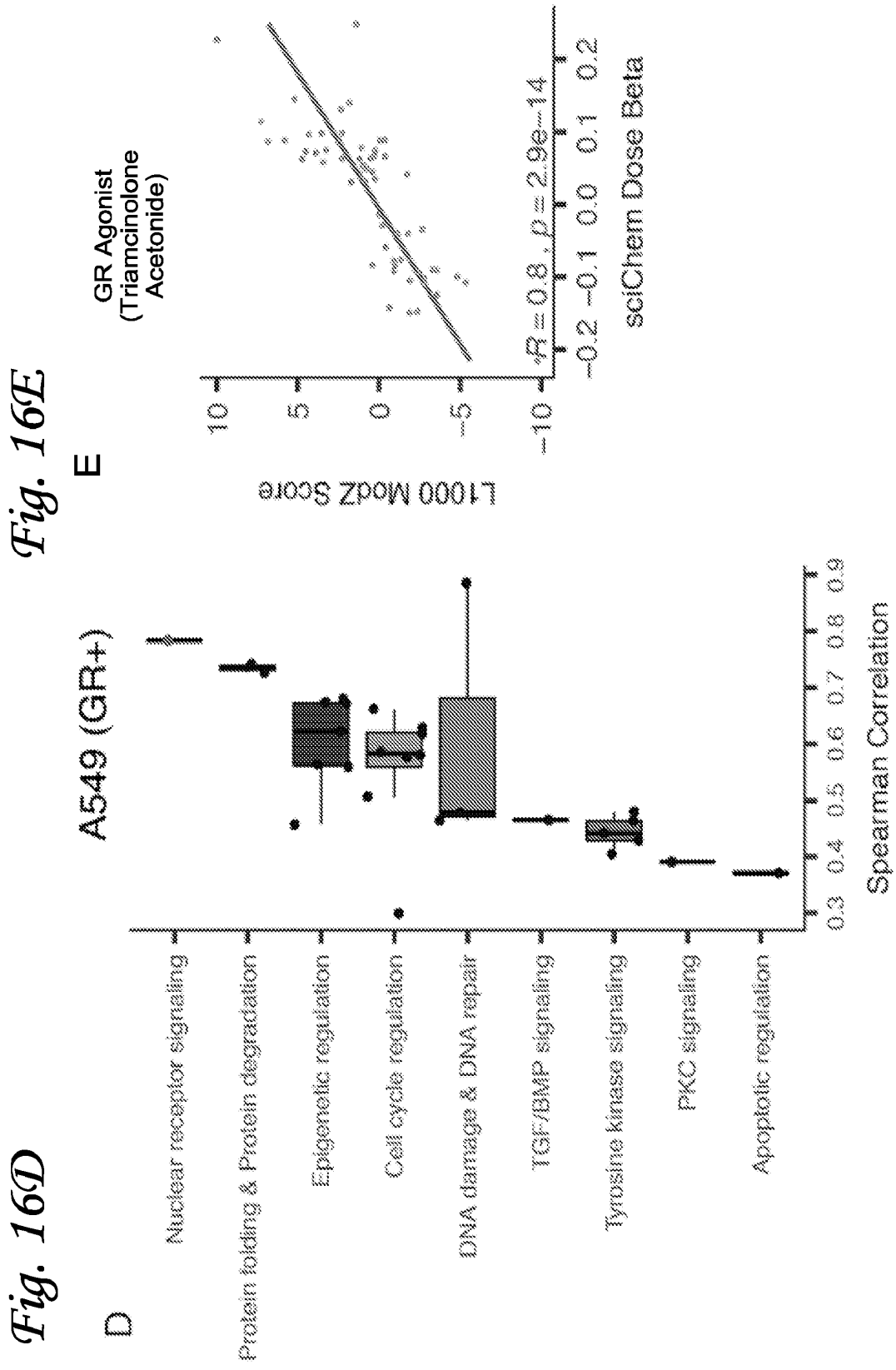


Fig. 16D

Fig. 16E

Fig. 17A

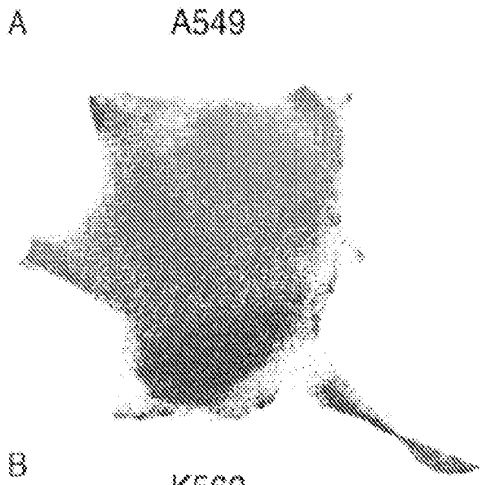


Fig. 17B



Fig. 17C

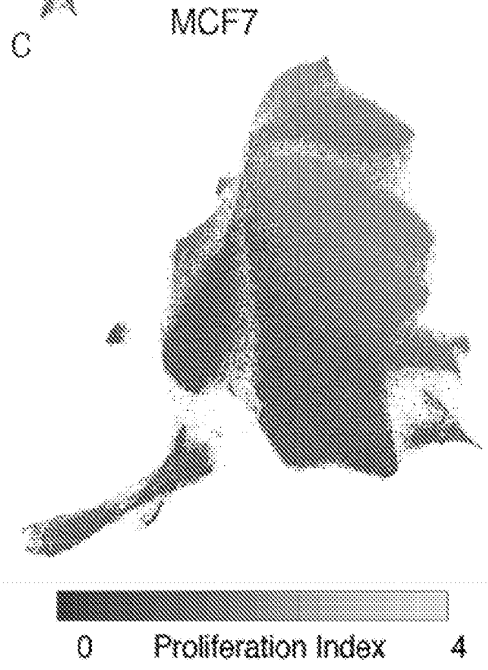


Fig. 17D

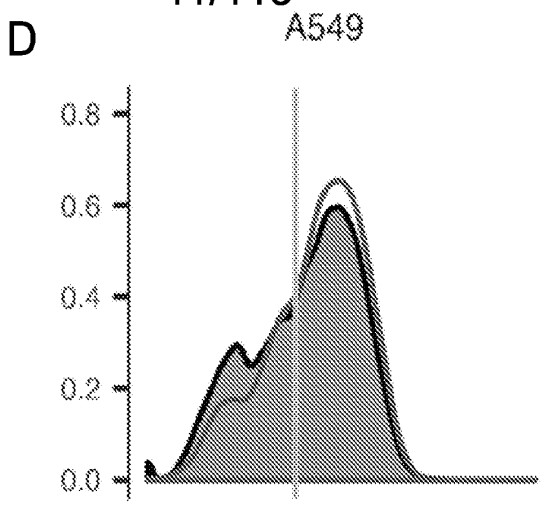


Fig. 17E

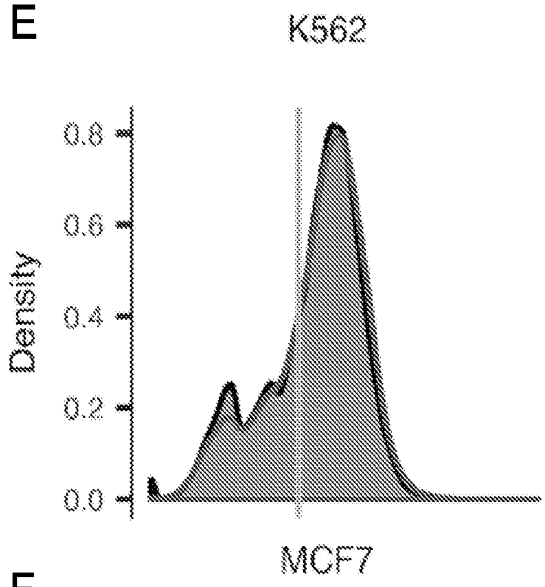


Fig. 17F

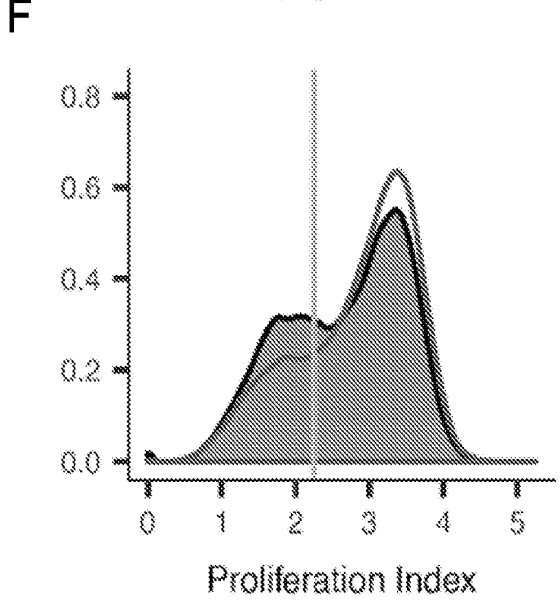


Fig. 17G

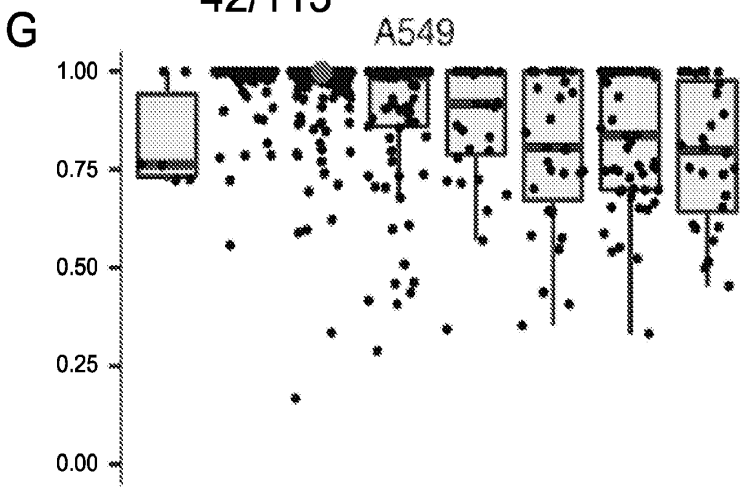


Fig. 17H

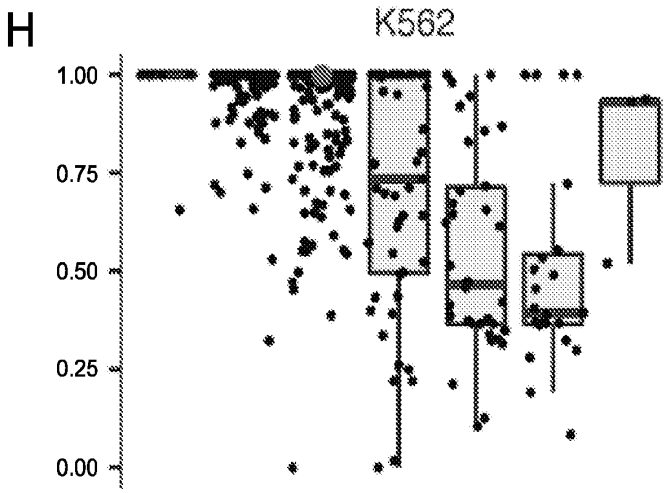
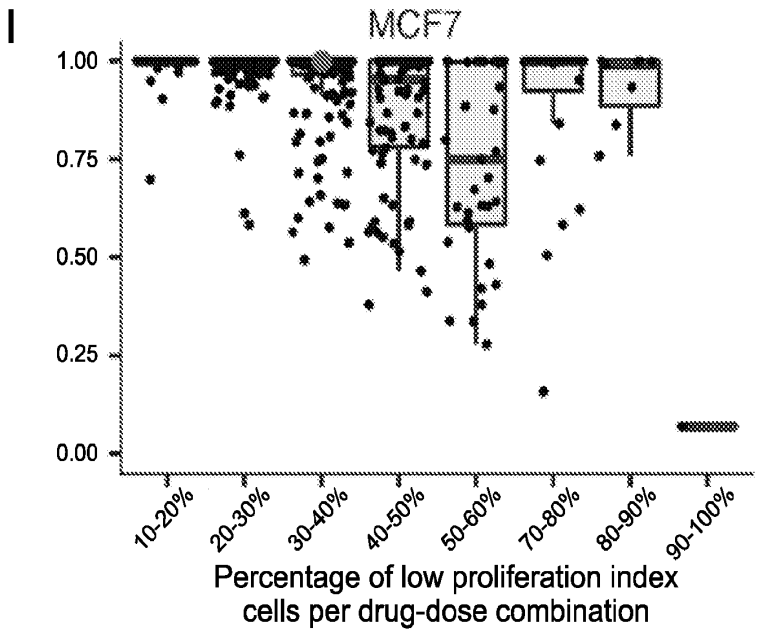


Fig. 17I



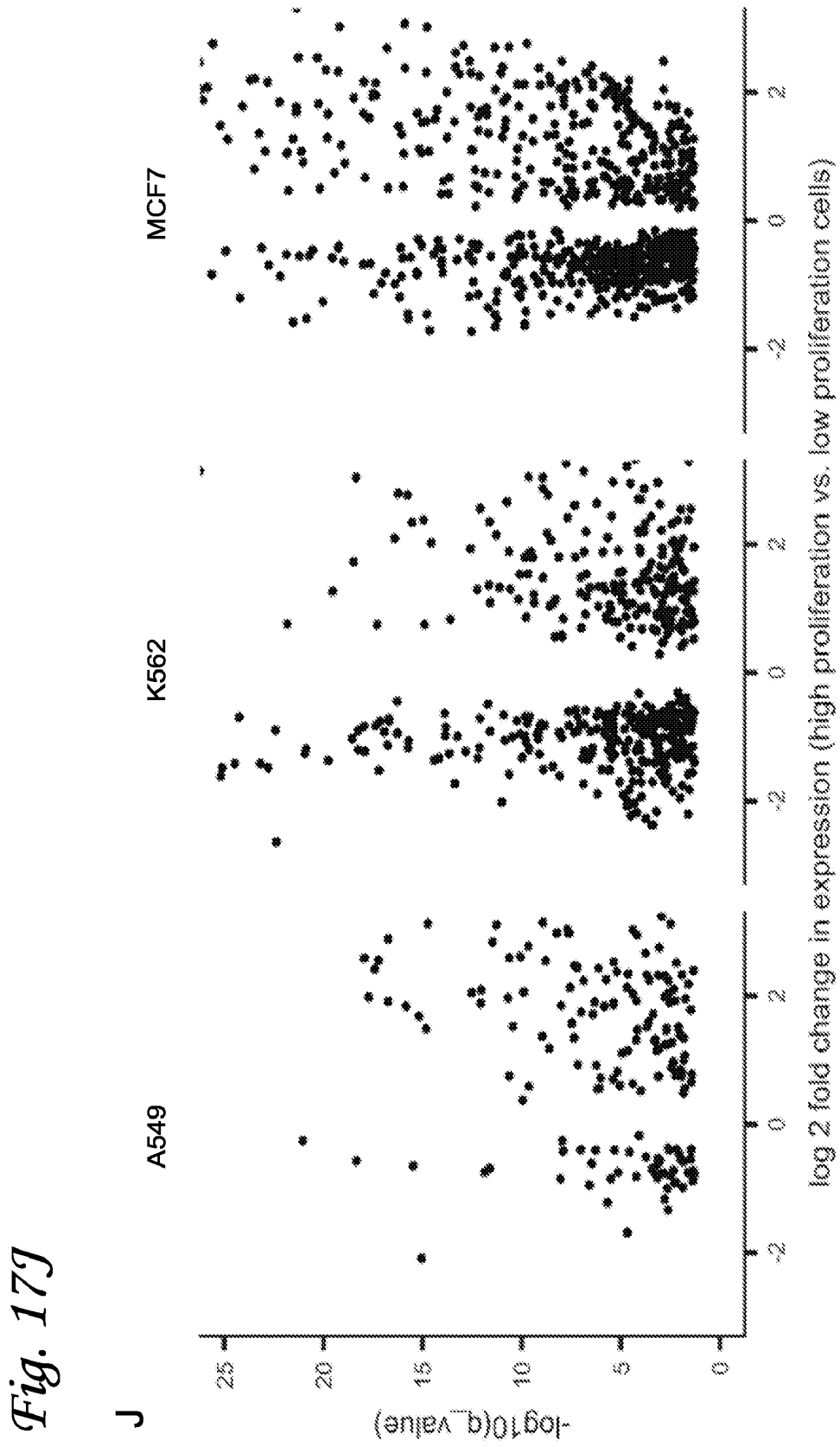

MCF7
K562
A549
log 2 fold change in expression (high proliferation vs. low proliferation cells)
-log10(q_value)
25
20
15
10
5
0
-2
0
2

Fig. 17J

J

Fig. 18A

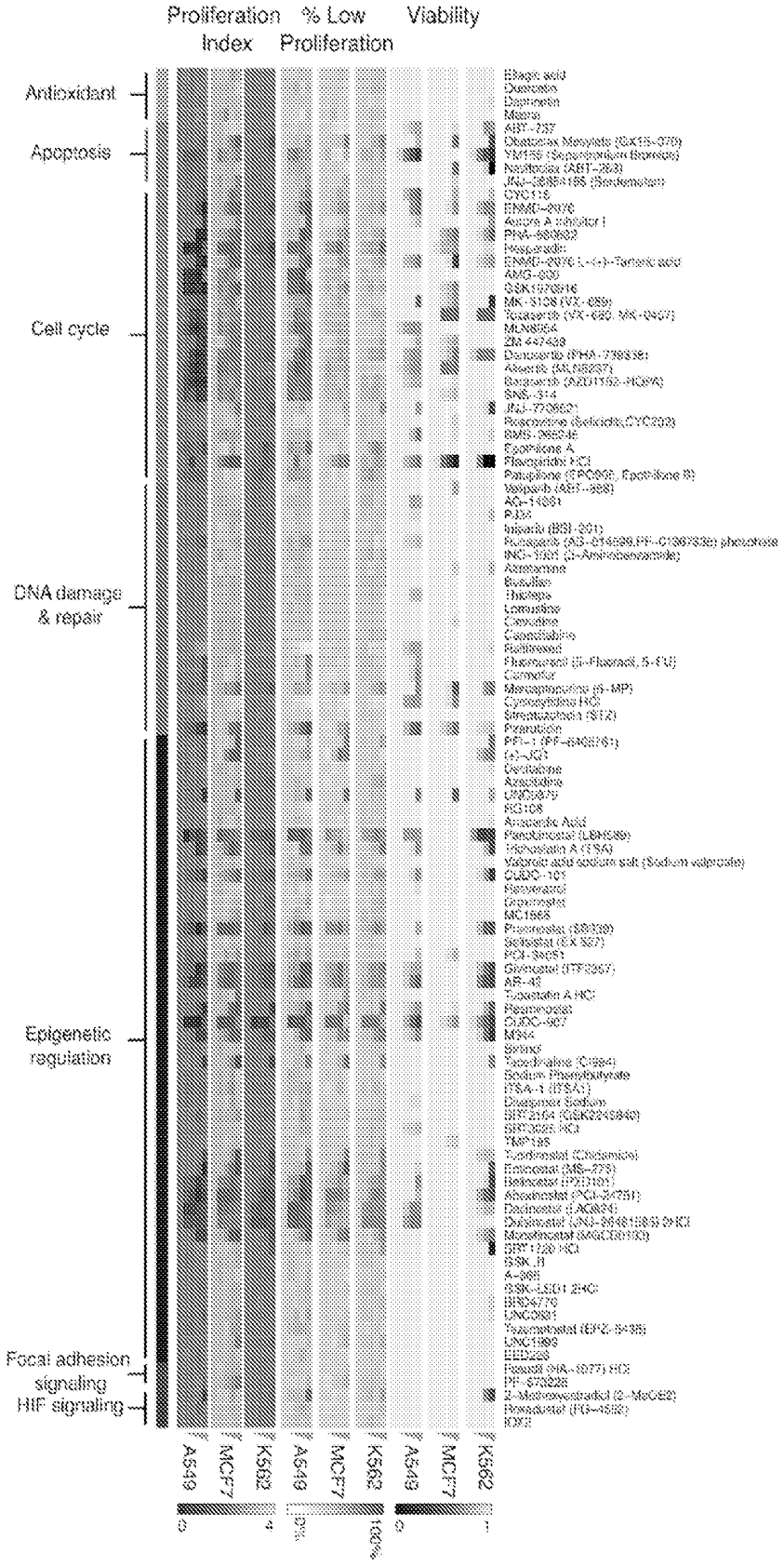

Proliferation Index
% Low Proliferation
Viability
Antioxidant
Apoptosis
Cell cycle
DNA damage & repair
Epigenetic regulation
Focal adhesion signaling
HIF signaling
A549
MCF7
K562
0
4
0%
100%
0
1

Fig. 18B

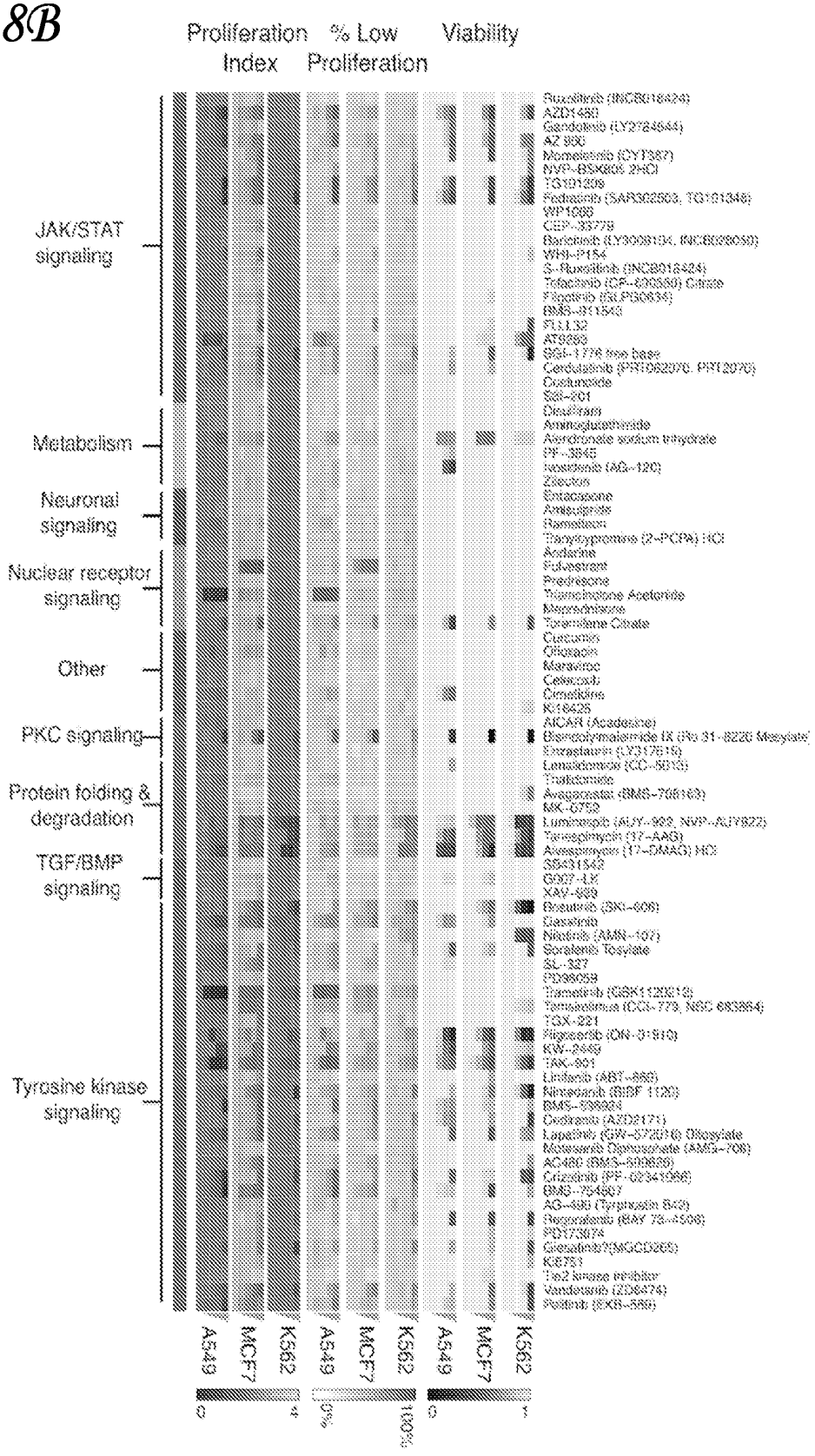

Proliferation Index
% Low Proliferation
Viability
JAK/STAT signaling
Metabolism
Neuronal signaling
Nuclear receptor signaling
Other
PKC signaling
Protein folding & degradation
TGF/BMP signaling
Tyrosine kinase signaling
Ruxolitinib (INCB018424)
AZD1480
Gandotinib (LY2784544)
AZ 960
Momelotinib (CYT387)
NVP-BSK805 2HCl
TG101209
Fedratinib (SAR302503, TG101348)
WP1066
CEP-33779
Baricitinib (LY3009104, INCB028050)
WHI-P154
S-Ruxolitinib (INCB018424)
Tofacitinib (CP-690550) Citrate
Pacritinib (SB1518)
BMS-911543
FLLL32
AT9283
BSI-1778 free base
Cerdulatinib (PRT062070, PRT2070)
Cucurbitacin
Sal-201
Disulfiram
Aminoglutethimide
Alendronate sodium trihydrate
PF-3845
Ibrutinib (AG-120)
Zileuton
Entacapone
Amisulpride
Ramelteon
Tranylcypromine (2-PCPA) HCl
Acalabrutinib
Fulvestrant
Prednisone
Triamcinolone Acetonide
Mesoridazine
Toremifene Citrate
Curcumin
Olaparib
Maraviroc
Celecoxib
Cimetidine
K118425
AICAR (Acadesine)
Bisindolylmaleimide IX (Ro 31-8220 Mesylate)
Enzastaurin (LY317615)
Lenalidomide (CC-5013)
Thalidomide
Avagacestat (BMS-708163)
MK-0752
Luminespib (AUY-922, NVP-AUY922)
Tanespimycin (17-AAG)
Alvespimycin (17-DMAG) HCl
SB431542
GW7647
XAV-939
Bosutinib (SKI-606)
Dasatinib
Nilotinib (AMN-107)
Sorafenib Tosylate
SL-327
PD98059
Trametinib (GSK1120212)
Temsirolimus (CCI-779, NSC 683864)
TGX-221
Ridaforolimus (ON-01910)
KW-2449
TAK-901
Linifanib (ABT-869)
Nintedanib (BIBF 1120)
BMS-599626
Dasatinib (AZD0171)
Lapatinib (GW-572016) Ditosylate
Motesanib Diphosphate (AMG-706)
AC480 (BMS-599626)
Crizotinib (PF-02341066)
BMS-754807
AG-490 (Tyrphostin B42)
Regorafenib (BAY 73-4506)
PD173074
Gefitinib?(MGCD265)
KIN001-135
Tie2 kinase inhibitor
Vandetanib (ZD6474)
Foretinib (EXEL-2880)
A549
MCF7
K562
0
1
10%
100%

Fig. 19A

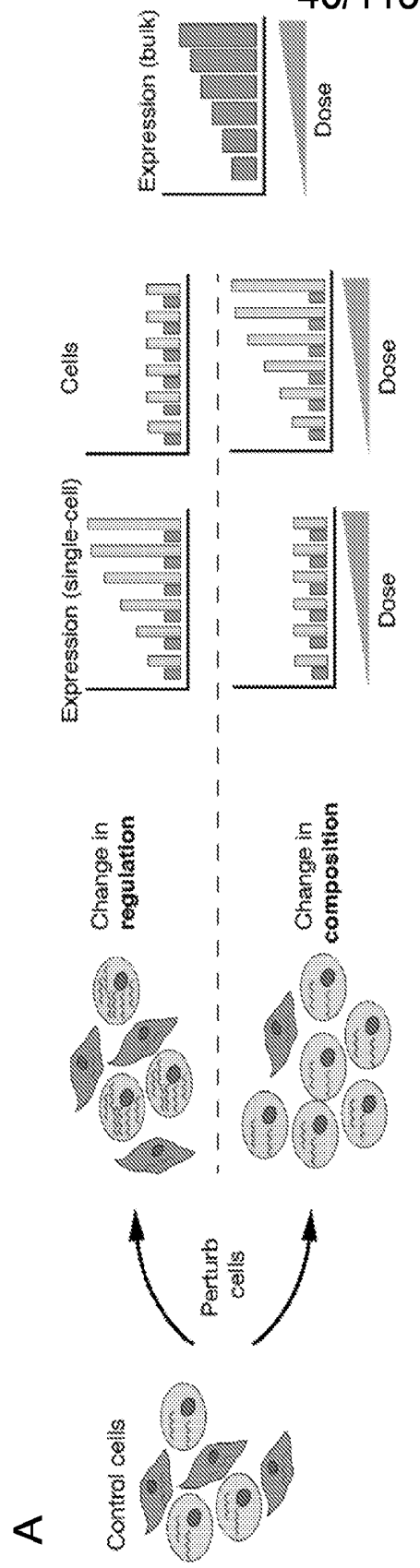

A
Control cells
Perturb cells
Change in regulation
Change in composition
Expression (single-cell)
Cells
Dose
Dose
Dose
Expression (bulk)
Dose

Fig. 19B

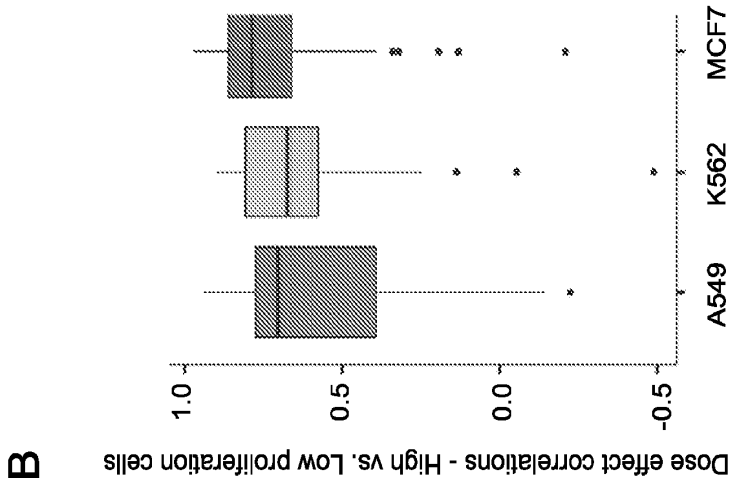


Fig. 19C

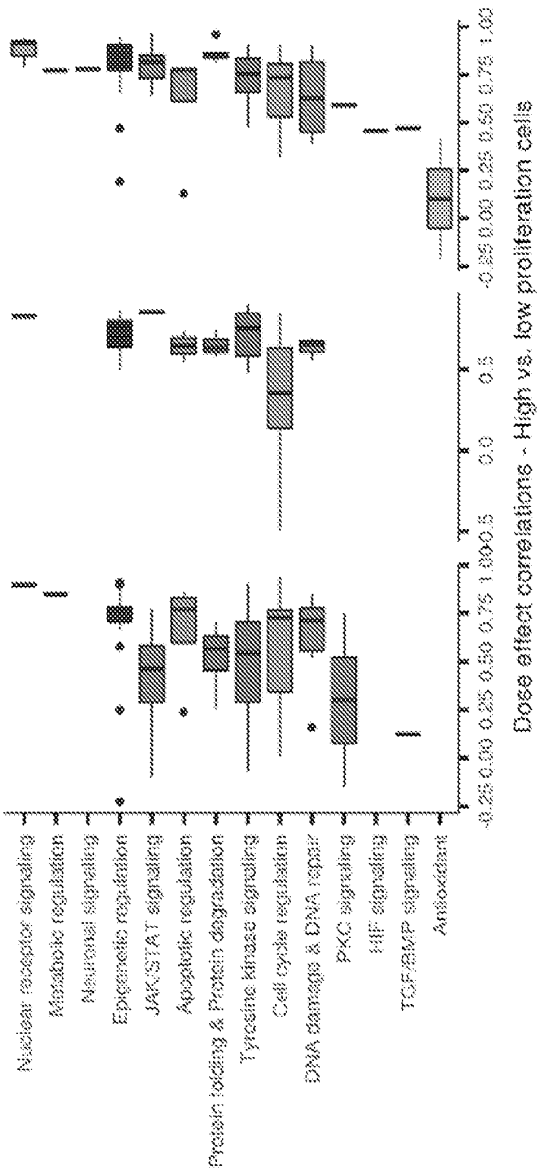
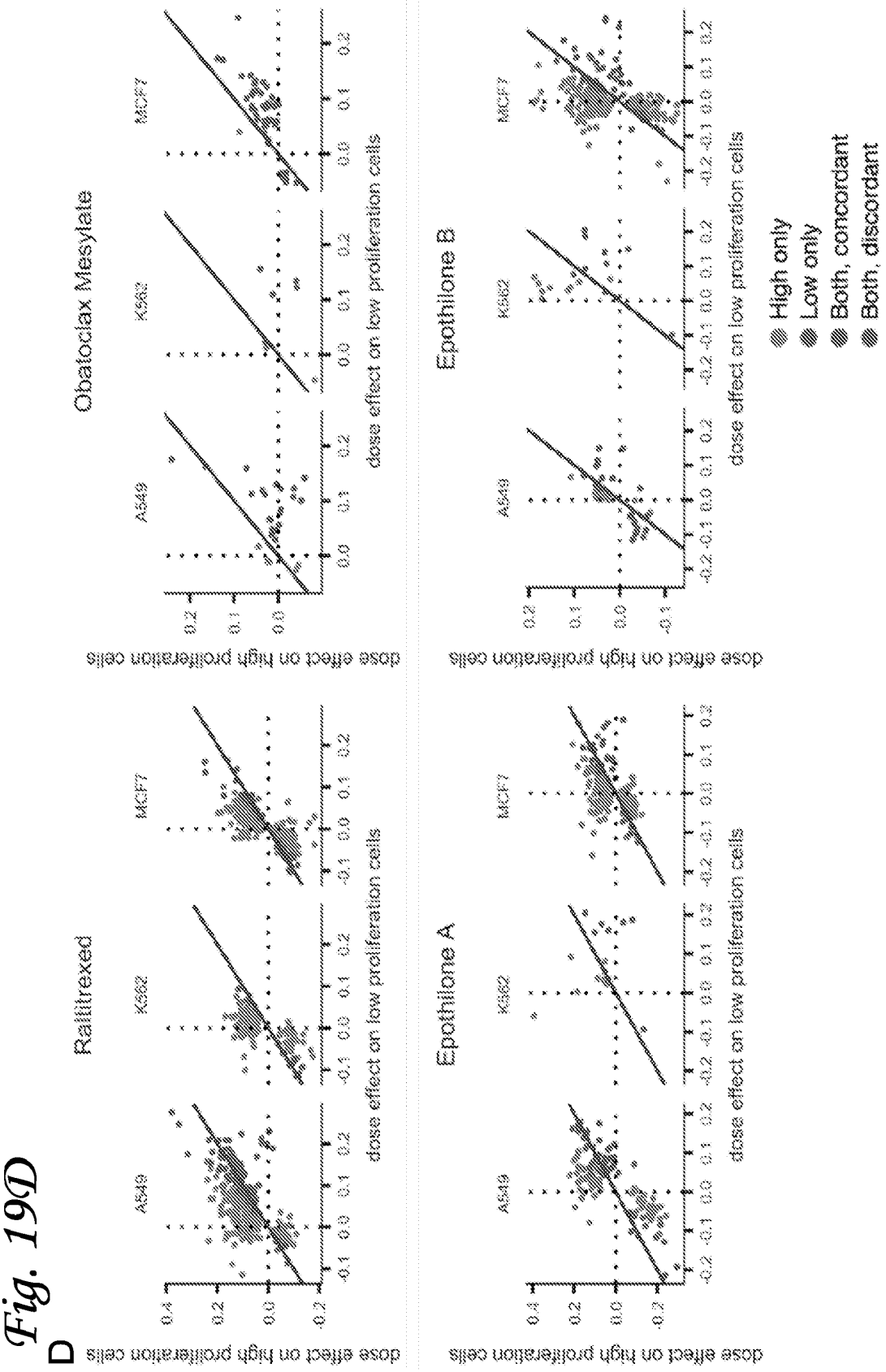


Fig. 19D


D
Raltitrexed
Obatoclax Mesylate
Epothilone A
Epothilone B
A549
K562
MCF7
dose effect on high proliferation cells
dose effect on low proliferation cells
High only
Low only
Both, concordant
Both, discordant

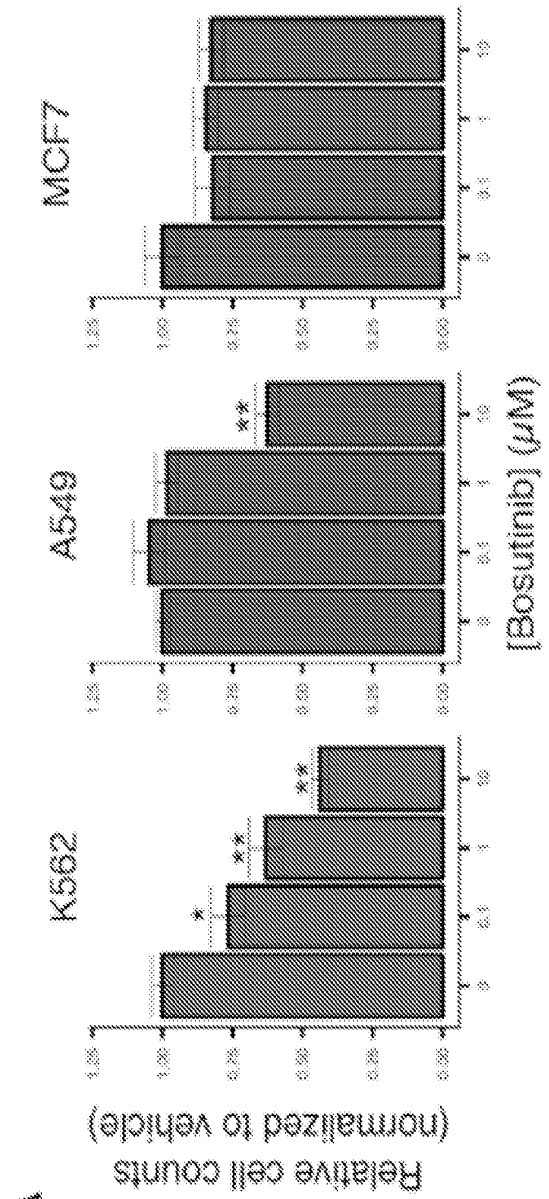


Fig. 20A

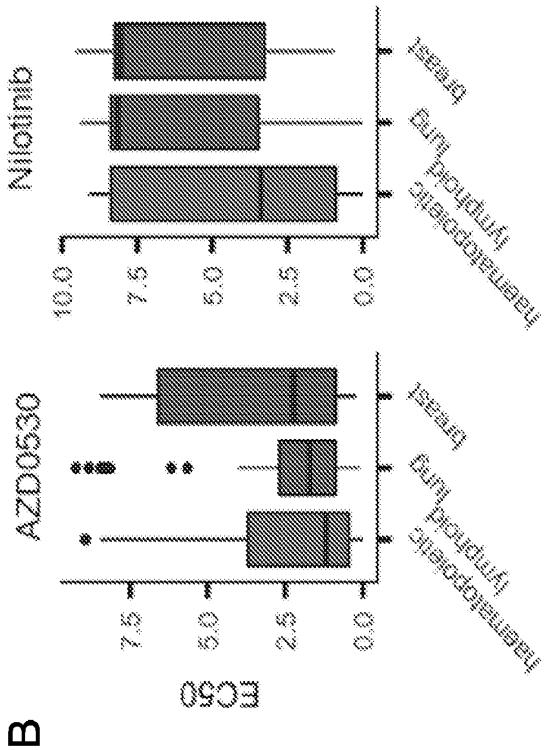


Fig. 20B

Fig. 20C

C

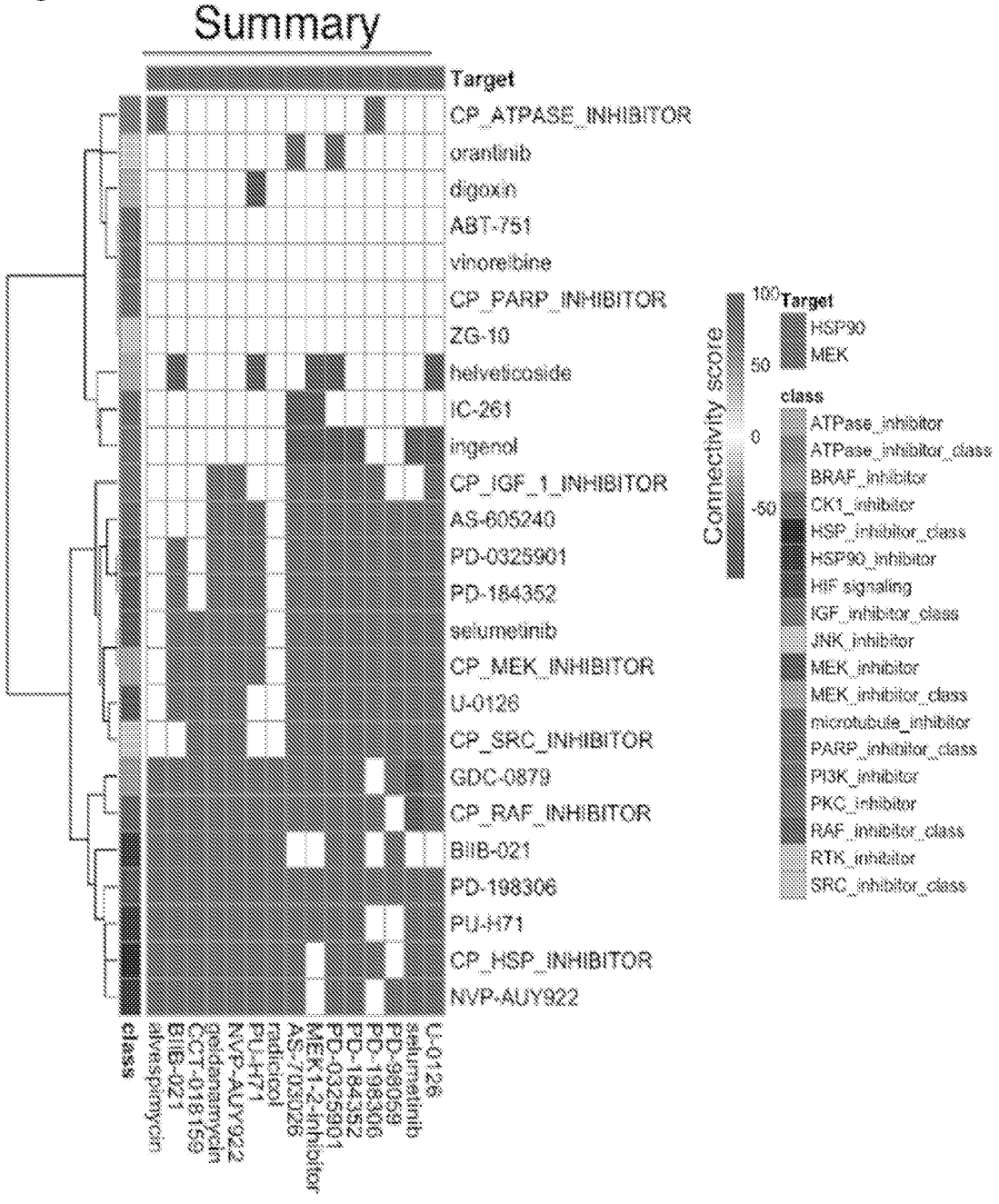

Summary
Target
CP_ATPASE_INHIBITOR
oranlinib
digoxin
ABT-751
vinorelbine
CP_PARP_INHIBITOR
ZG-10
helveticoside
IC-261
ingenol
CP_IGF_1_INHIBITOR
AS-605240
PD-0325901
PD-184352
selumetinib
CP_MEK_INHIBITOR
U-0126
CP_SRC_INHIBITOR
GDC-0879
CP_RAF_INHIBITOR
BIIB-021
PD-198306
PU-H71
CP_HSP_INHIBITOR
NVP-AUY922
class
alvespimycin
BIIB-021
CCT-018159
geldanamycin
NVP-AUY922
PU-H71
radicicol
AS-703026
MEK1-2-inhibitor
PD-0325901
PD-184352
PD-198306
PD-98059
selumetinib
U-0126
Connectivity score
100
50
0
-50
Target
HSP90
MEK
class
ATPase_inhibitor
ATPase_inhibitor_class
BRAF_inhibitor
CK1_inhibitor
HSP_inhibitor_class
HSP90_inhibitor
HIF signaling
IGF_inhibitor_class
JNK_inhibitor
MEK_inhibitor
MEK_inhibitor_class
microtubule_inhibitor
PARP_inhibitor_class
PI3K_inhibitor
PKC_inhibitor
RAF_inhibitor_class
RTK_inhibitor
SRC_inhibitor_class

Fig. 20D

D

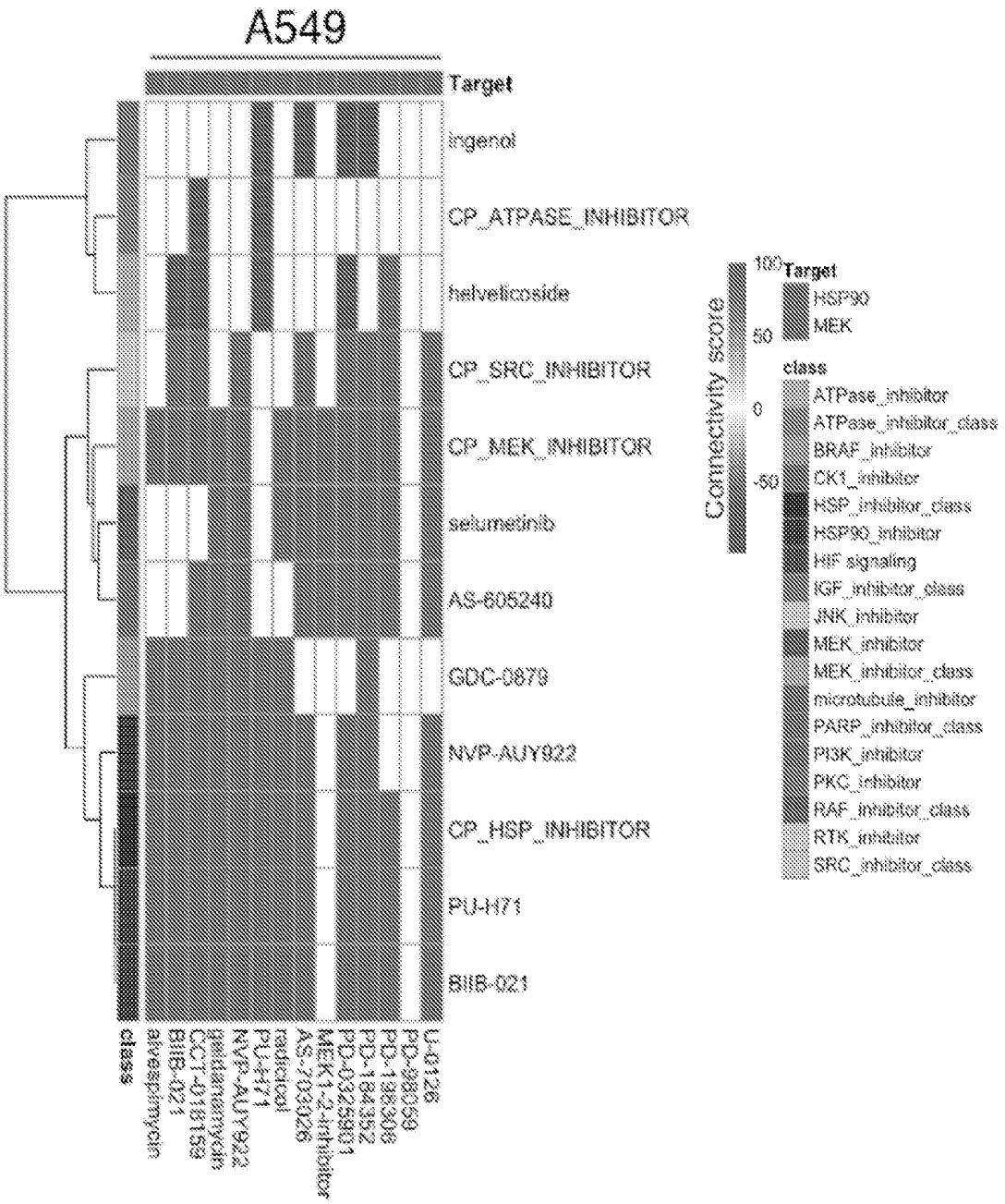

A549
Target
Ingenol
CP_ATPASE_INHIBITOR
helveticoside
CP_SRC_INHIBITOR
CP_MEK_INHIBITOR
selumetinib
AS-605240
GDC-0879
NVP-AUY922
CP_HSP_INHIBITOR
PU-H71
BIIB-021
class
alvespimycin
BIIB-021
CCT-018159
geldanamycin
NVP-AUY922
PU-H71
radicicol
AS-703026
MEK1-2-inhibitor
PD-032591
PD-184352
PD-188308
PD-98059
U-0126
Connectivity score
100
50
0
-50
Target
HSP90
MEK
class
ATPase_inhibitor
ATPase_inhibitor_class
BRAF_inhibitor
CK1_inhibitor
HSP_inhibitor_class
HSP90_inhibitor
HIF signaling
IGF_inhibitor_class
JNK_inhibitor
MEK_inhibitor
MEK_inhibitor_class
microtubule_inhibitor
PARP_inhibitor_class
PI3K_inhibitor
PKC_inhibitor
RAF_inhibitor_class
RTK_inhibitor
SRC_inhibitor_class

Fig. 20E

E

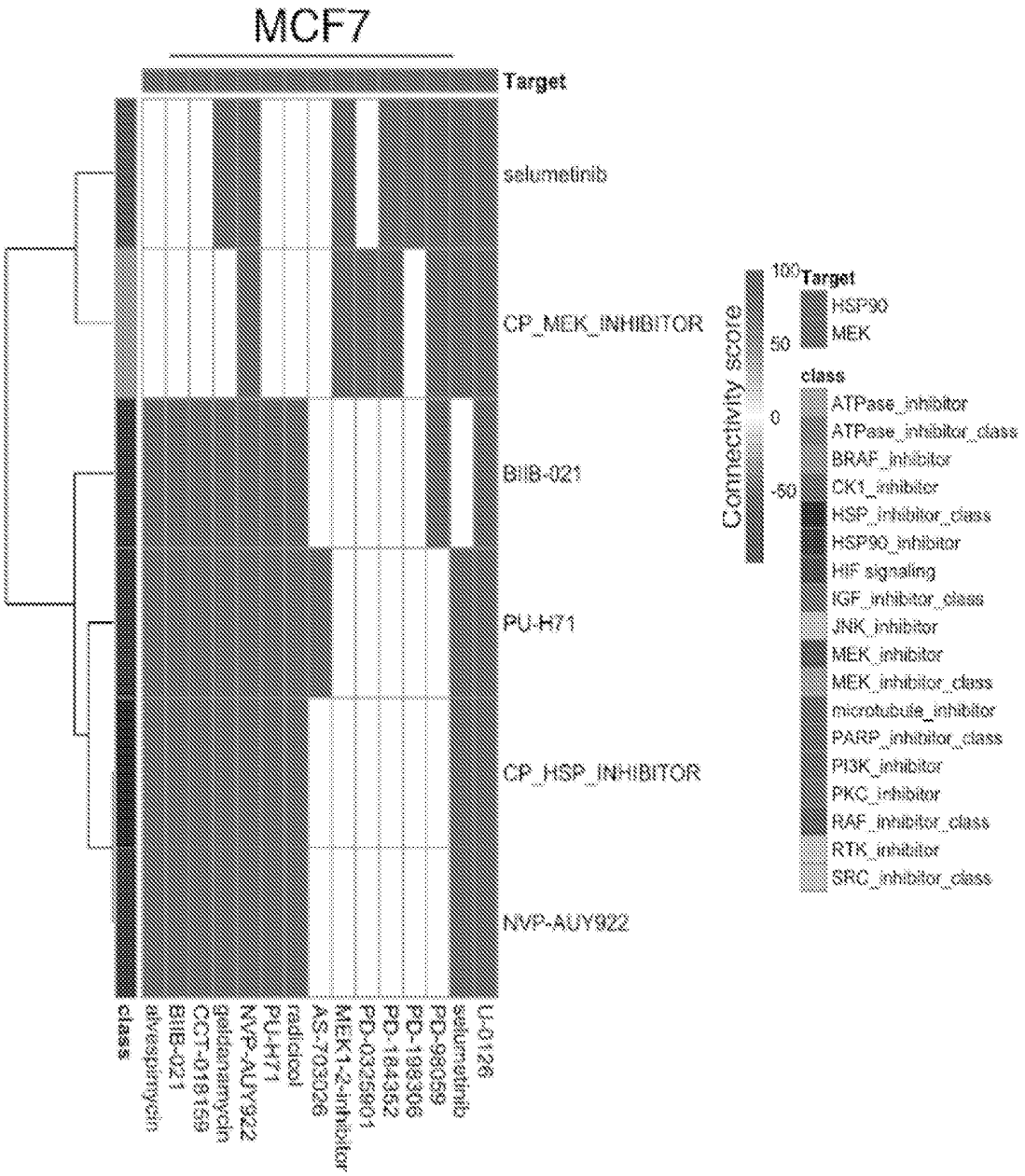

MCF7
Target
selumetinib
CP_MEK_INHIBITOR
BIIB-021
PU-H71
CP_HSP_INHIBITOR
NVP-AUY922
class
alvespimycin
BIIB-021
CCT-018159
geldanamycin
NVP-AUY922
PU-H71
radicicol
AS-703026
MEK1-2-inhibitor
PD-0325901
PD-184352
PD-198306
PD-98059
selumetinib
U-0126
Connectivity score
100
50
0
-50
Target
HSP90
MEK
class
ATPase_inhibitor
ATPase_inhibitor_class
BRAF_inhibitor
CK1_inhibitor
HSP_inhibitor_class
HSP90_inhibitor
HIF signaling
IGF_inhibitor_class
JNK_inhibitor
MEK_inhibitor
MEK_inhibitor_class
microtubule_inhibitor
PARP_inhibitor_class
PI3K_inhibitor
PKC_inhibitor
RAF_inhibitor_class
RTK_inhibitor
SRC_inhibitor_class

Fig. 21

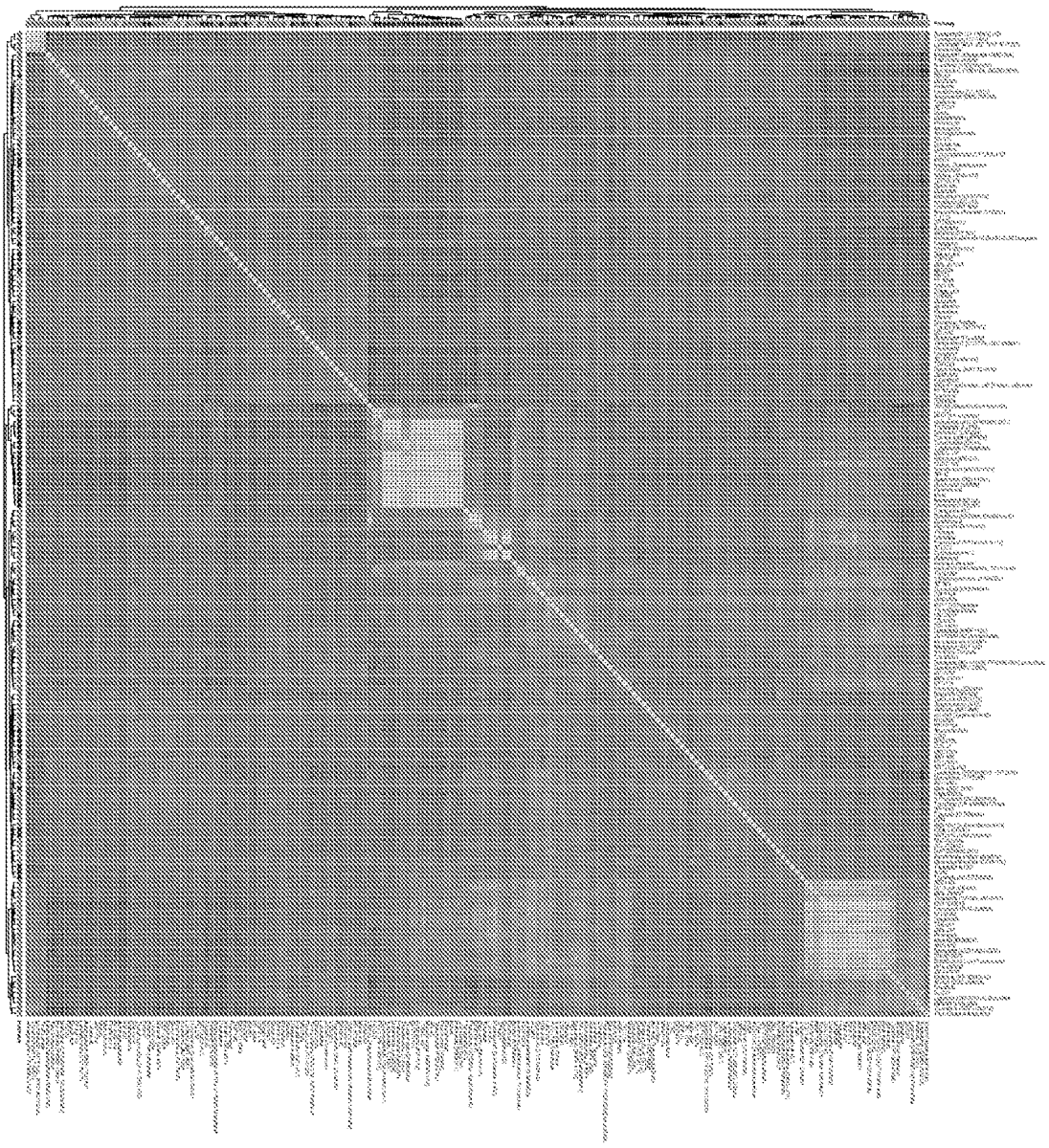


Fig. 22

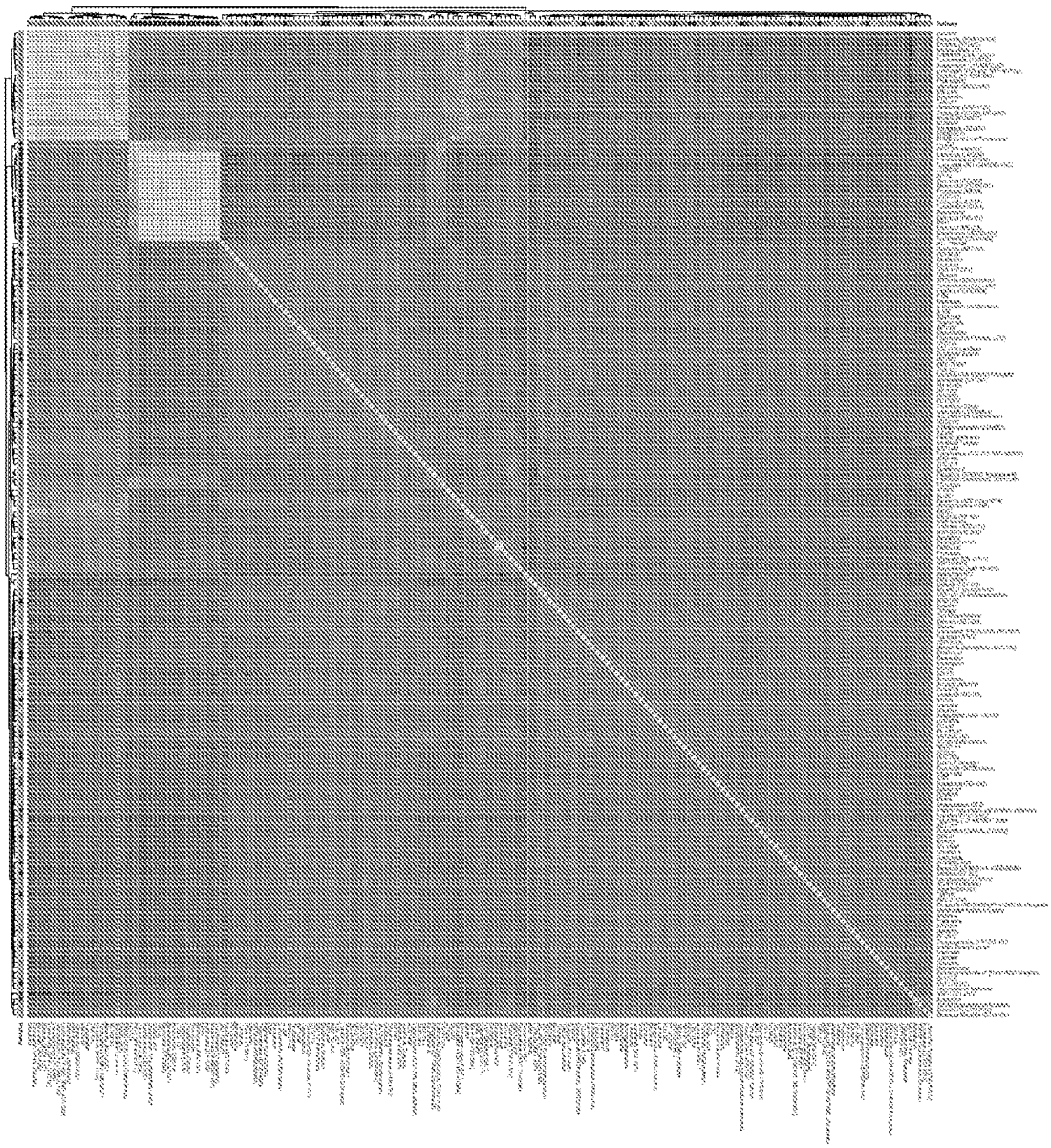


Fig. 23

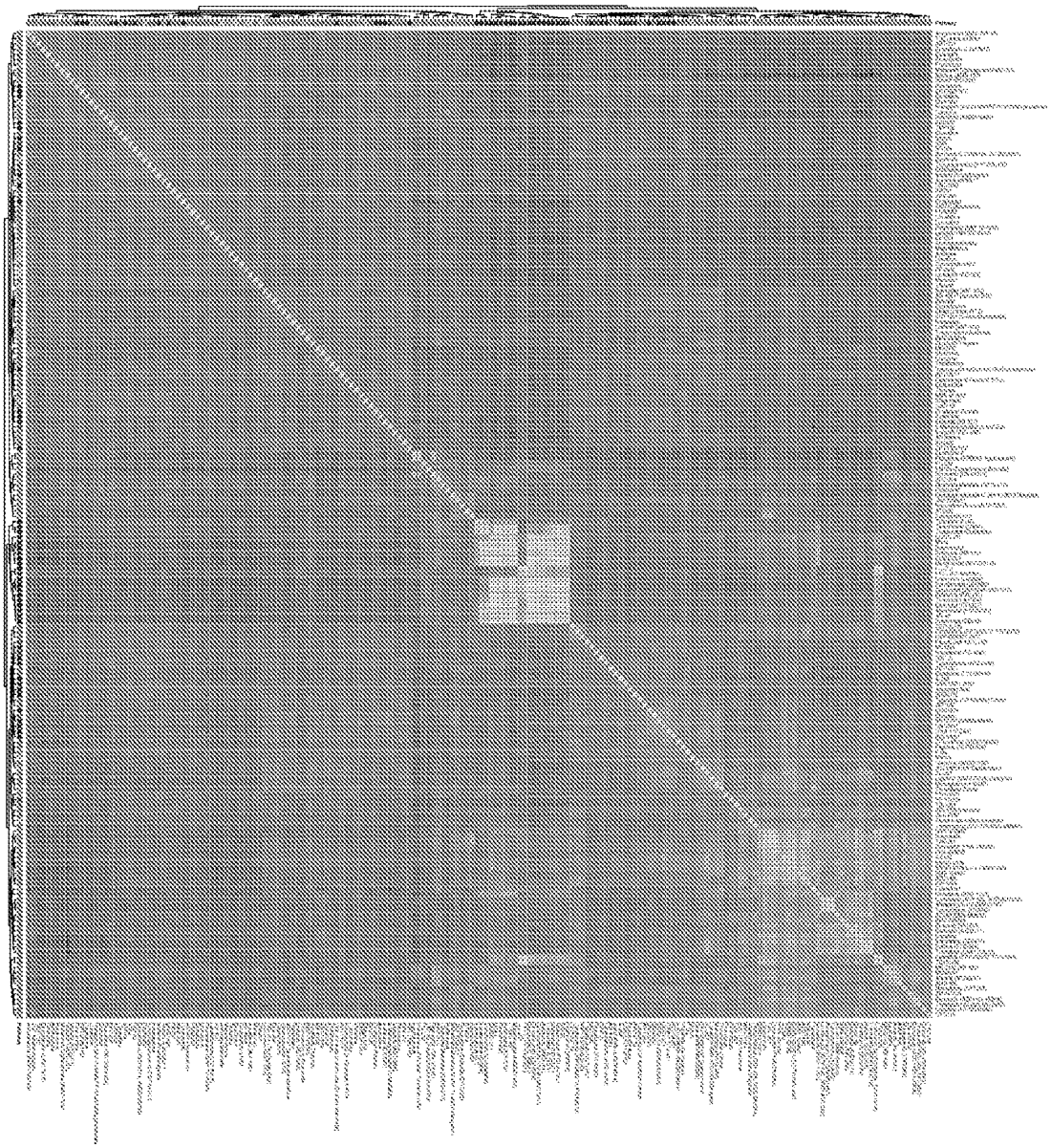


Fig. 24A

A

A549

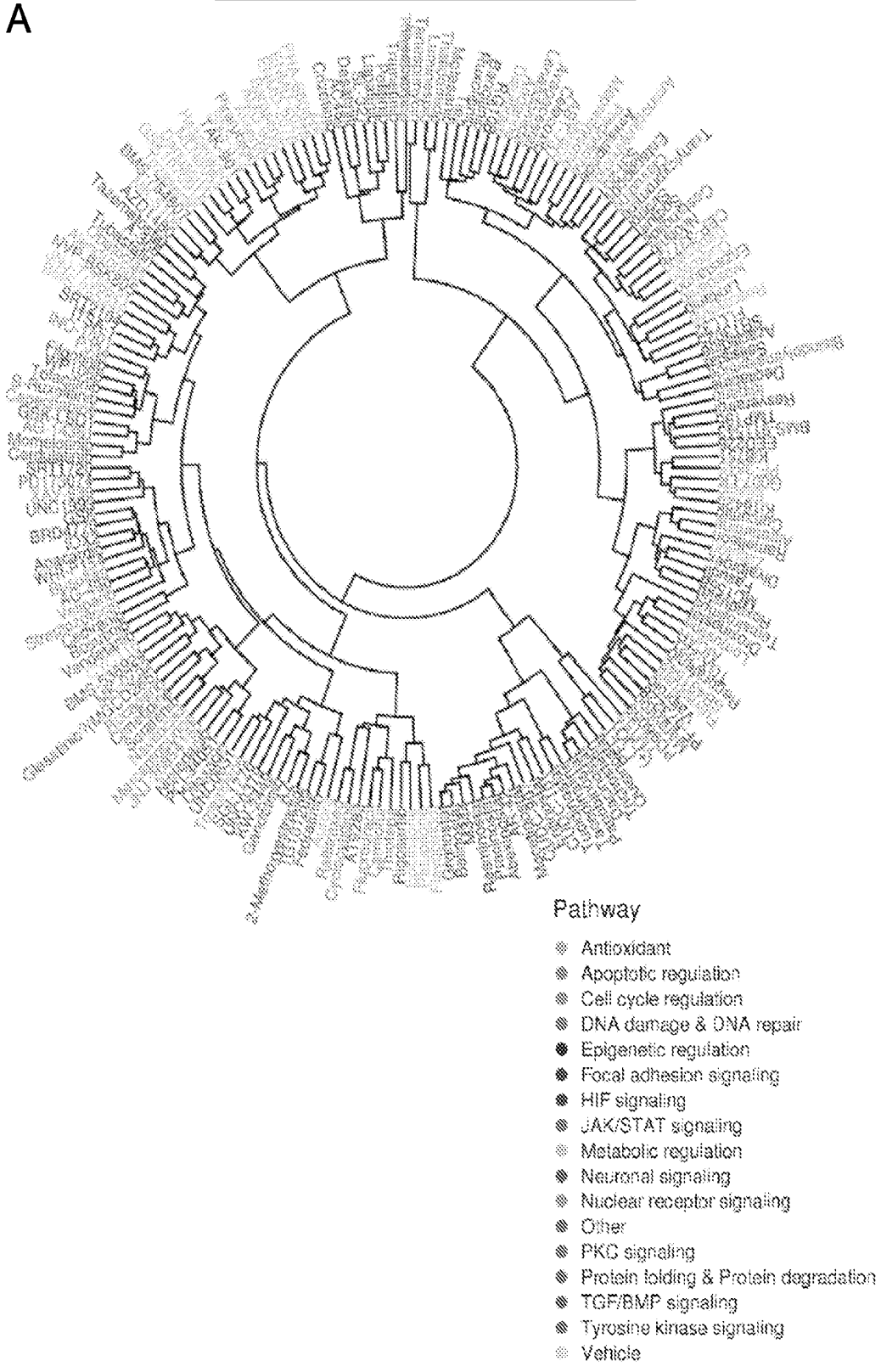


Fig. 24B

B

K562

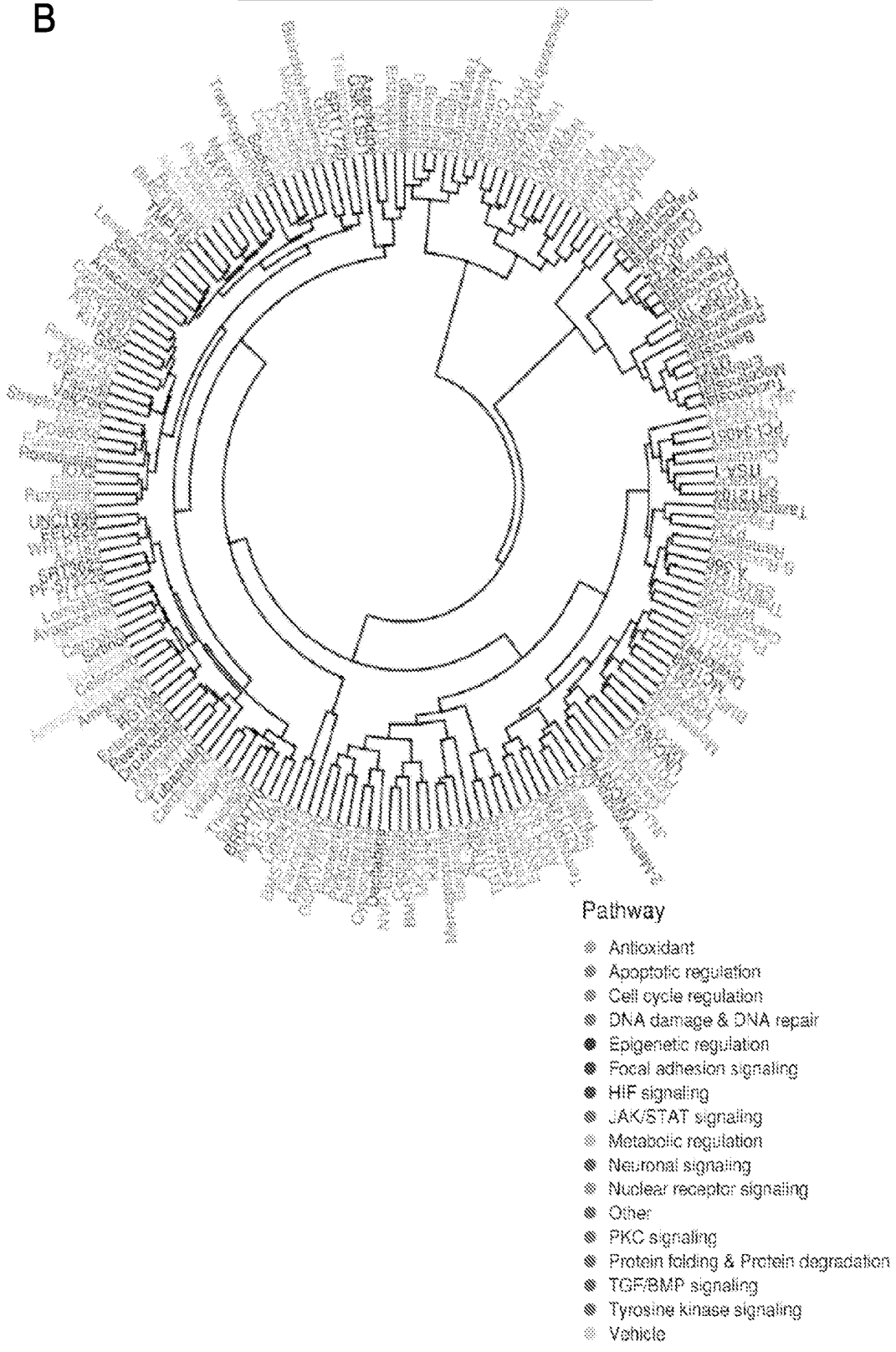


Fig. 24C

C

MCF7

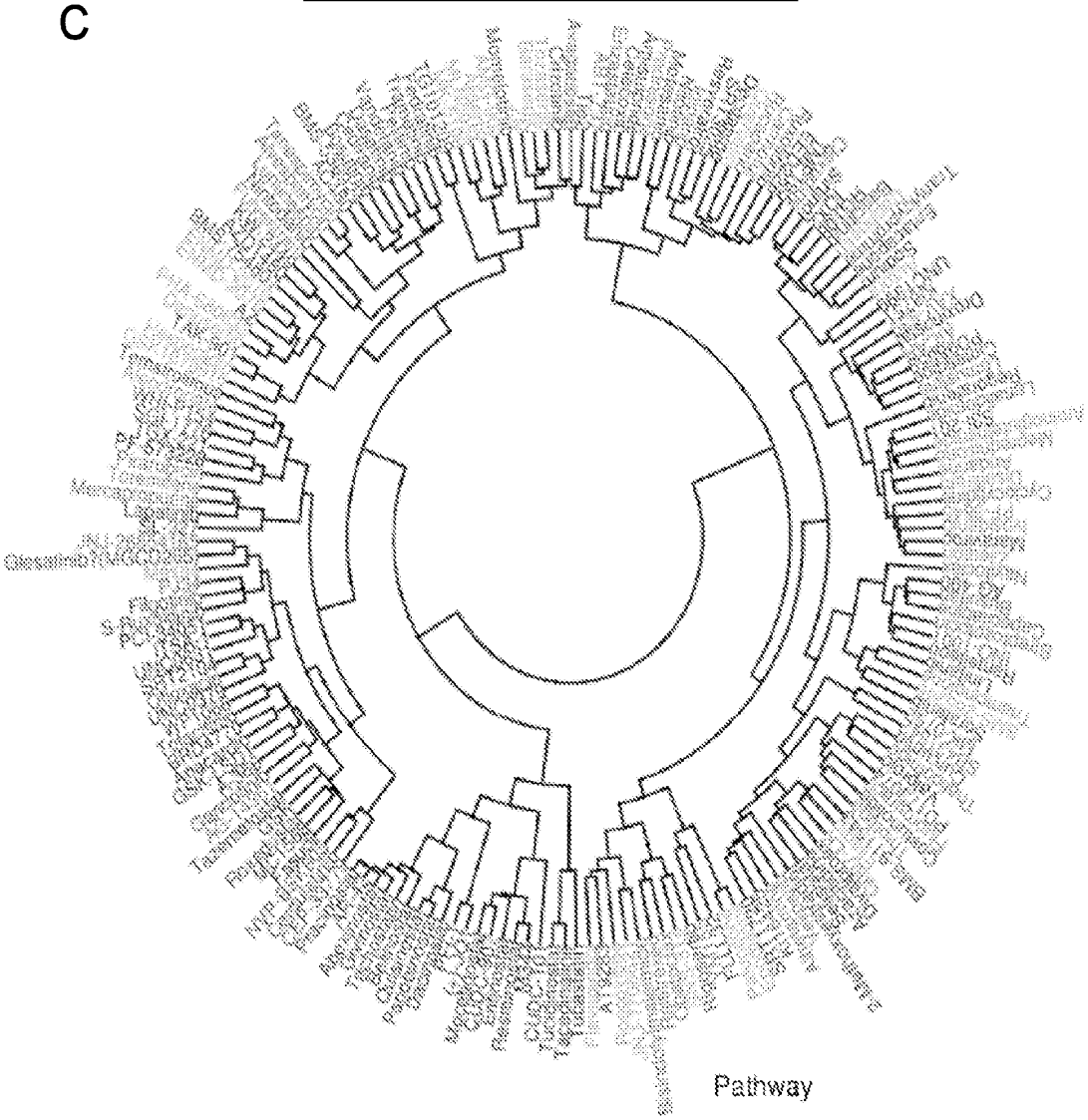
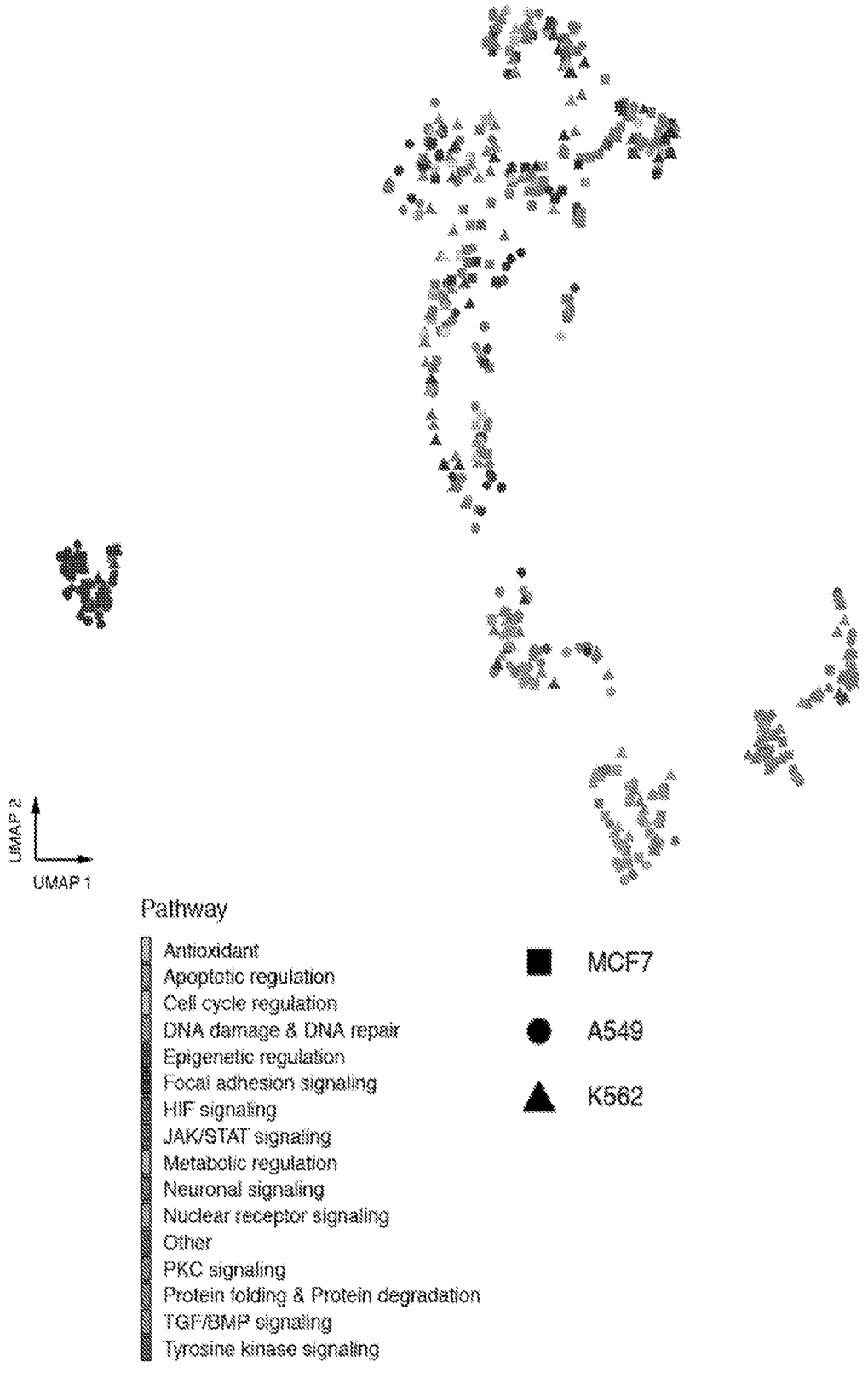


Fig. 25


UMAP 2
UMAP 1
Pathway
Antioxidant
Apoptotic regulation
Cell cycle regulation
DNA damage & DNA repair
Epigenetic regulation
Focal adhesion signaling
HIF signaling
JAK/STAT signaling
Metabolic regulation
Neuronal signaling
Nuclear receptor signaling
Other
PKC signaling
Protein folding & Protein degradation
TGF/BMP signaling
Tyrosine kinase signaling
MCF7
A549
K562

Figs. 26A, 26B

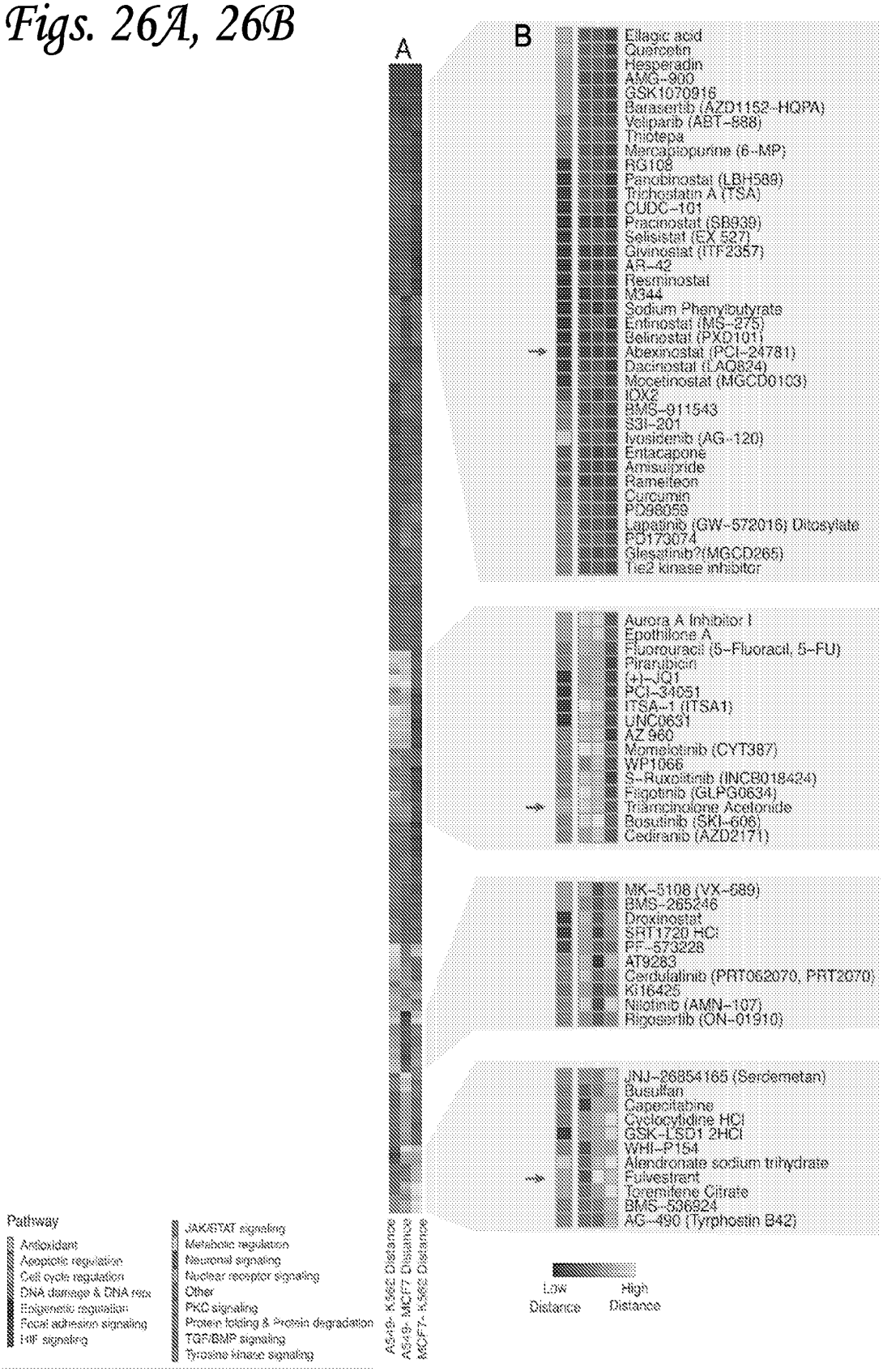

A
B
Ellagic acid
Quercetin
Hesperadin
AMG-900
GSK1070916
Barasertib (AZD1152-HQPA)
Veliparib (ABT-888)
Thiotepa
Mercaptopurine (6-MP)
RG108
Panobinostat (LBH589)
Trichostatin A (TSA)
CUDC-101
Pracinostat (SB939)
Selisistat (EX 527)
Givinostat (ITF2357)
AR-42
Resminostat
M344
Sodium Phenylbutyrate
Entinostat (MS-275)
Belinostat (PXD101)
Abexinostat (PCI-24781)
Dacinostat (LAQ824)
Mocetinostat (MGCD0103)
IDX2
BMS-911543
S3I-201
Iyosidenib (AG-120)
Entacapone
Amisulpride
Ramelteon
Curcumin
PD98059
Lapatinib (GW-572016) Ditosylate
PD173074
Glesatinib?(MGCD265)
Tie2 kinase inhibitor
Aurora A Inhibitor I
Epothilone A
Fluorouracil (5-Fluoracil, 5-FU)
Pirarubicin
(+)-JQ1
PCI-34051
ITSA-1 (ITSA1)
UNC0631
AZ 960
Momelotinib (CYT387)
WP1066
S-Ruxolitinib (INCB018424)
Filgotinib (GLPG0634)
Triamcinolone Acetonide
Bosutinib (SKI-606)
Cediranib (AZD2171)
MK-5108 (VX-689)
BMS-265246
Droxinostat
SRT1720 HCl
PF-573228
AT9283
Cerdulatinib (PRT062070, PRT2070)
KI16425
Nilotinib (AMN-107)
Rigosertib (ON-01910)
JNJ-26854165 (Serdemetan)
Busulfan
Capecitabine
Cyclocytidine HCl
GSK-LSD1 2HCl
WHI-P154
Alendronate sodium trihydrate
Fulvestrant
Toremifene Citrate
BMS-536924
AG-490 (Tyrphostin B42)
Low Distance
High Distance
Pathway
Antioxidant
Apoptotic regulation
Cell cycle regulation
DNA damage & DNA rep
Epigenetic regulation
Focal adhesion signaling
HIF signaling
JAK/STAT signaling
Metabolic regulation
Neuronal signaling
Nuclear receptor signaling
Other
PKC signaling
Protein folding & Protein degradation
TGF/BMP signaling
Tyrosine kinase signaling
A549- K562 Distance
A549- MCF7 Distance
MCF7- K562 Distance

Fig. 26C



Fig. 26D

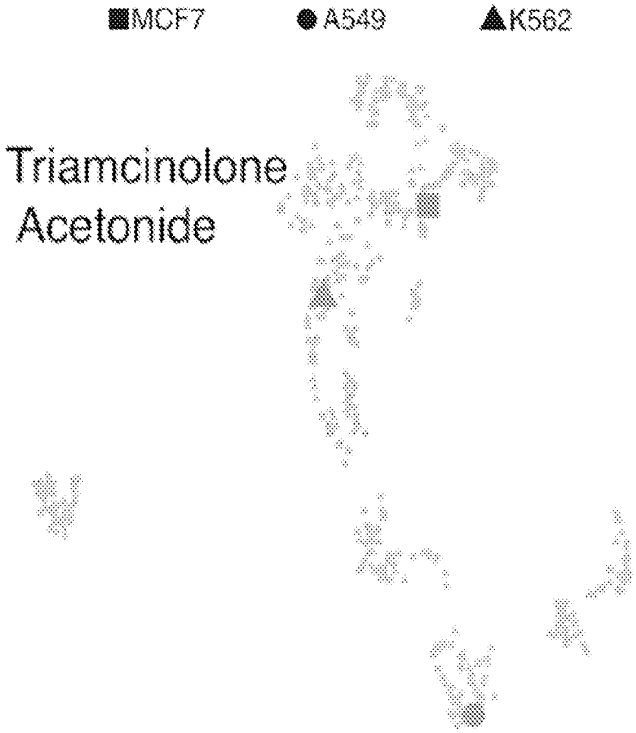


Fig. 26E

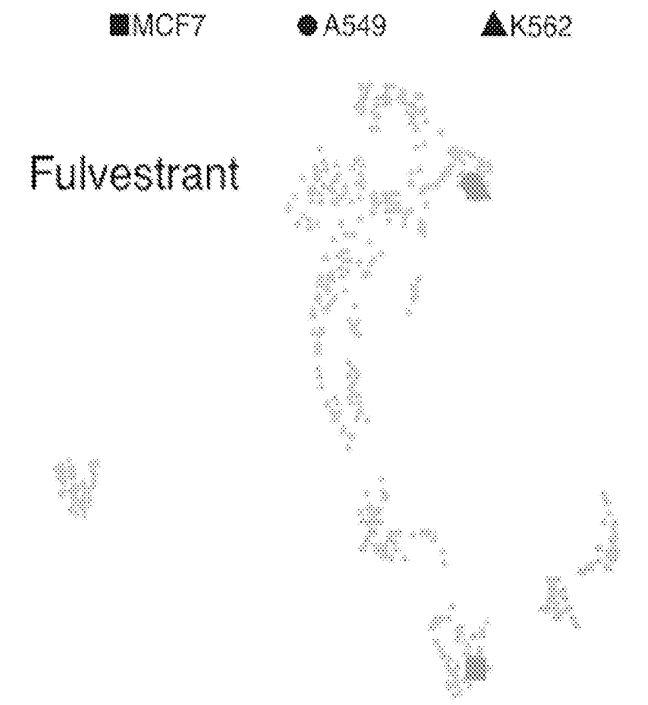


Fig. 26F

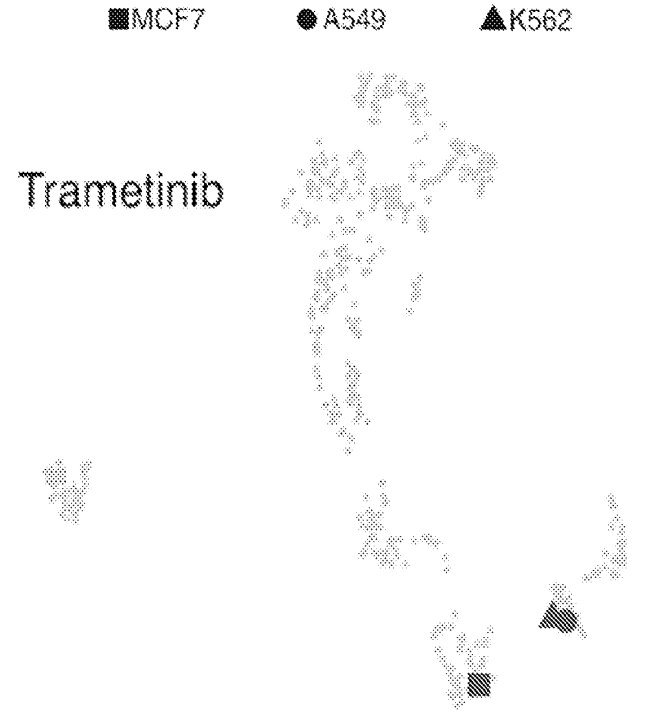


Fig. 27A

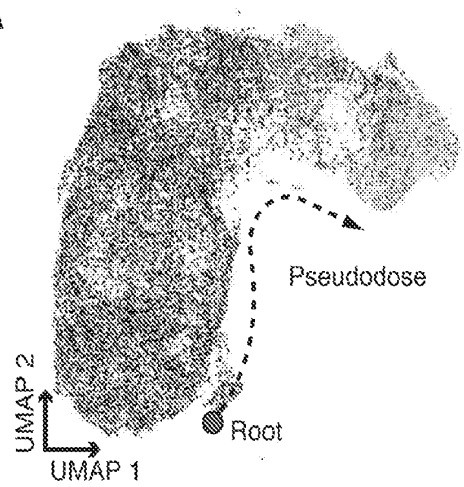


Fig. 27B

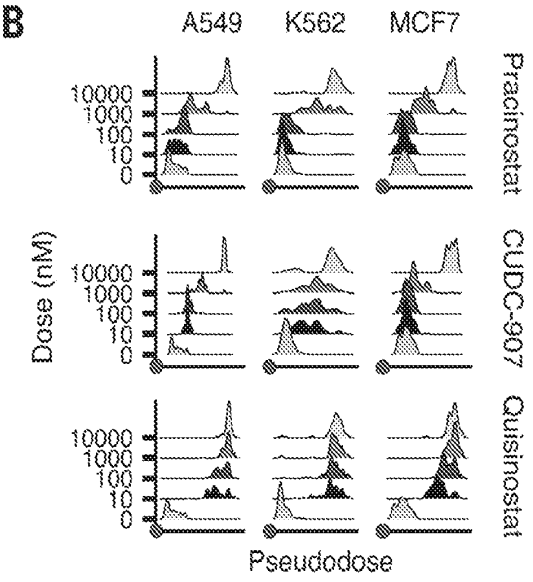


Fig. 27C

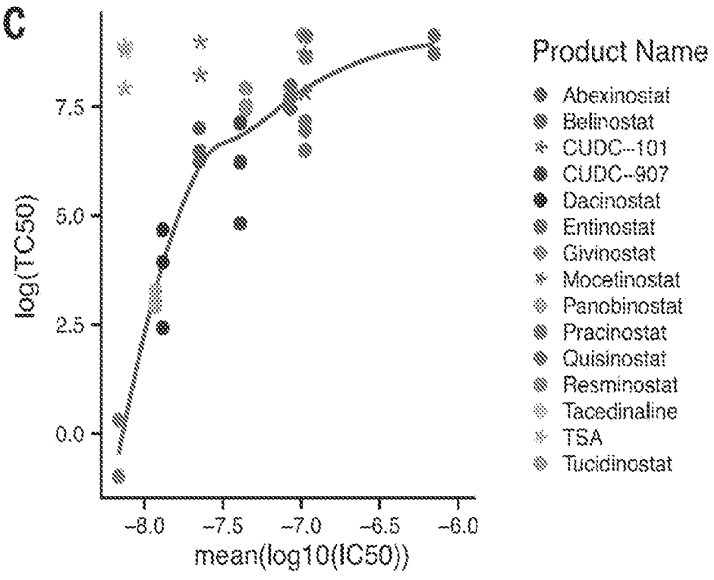


Fig. 28A

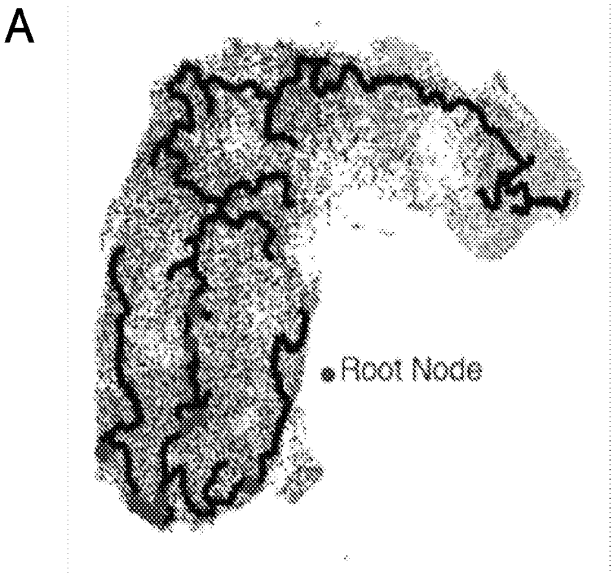


Fig. 28B



Fig. 28C

C

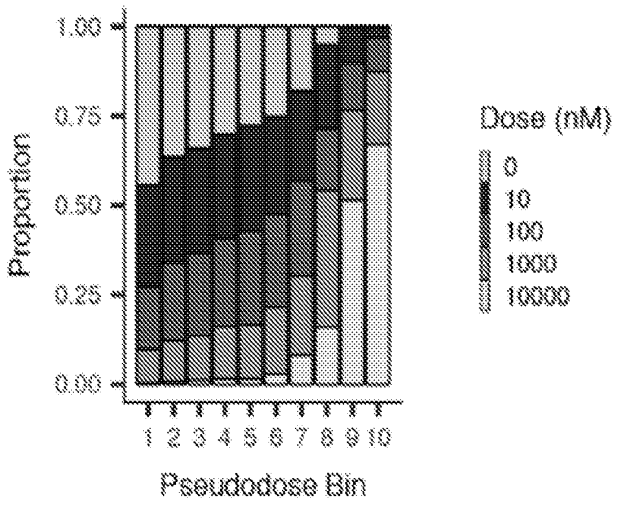


Fig. 28D

D

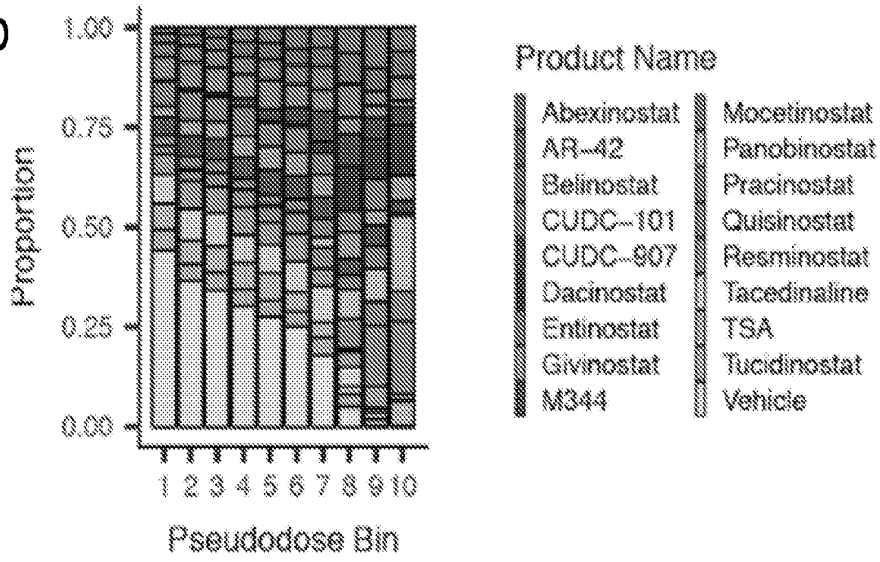


Fig. 28E

E

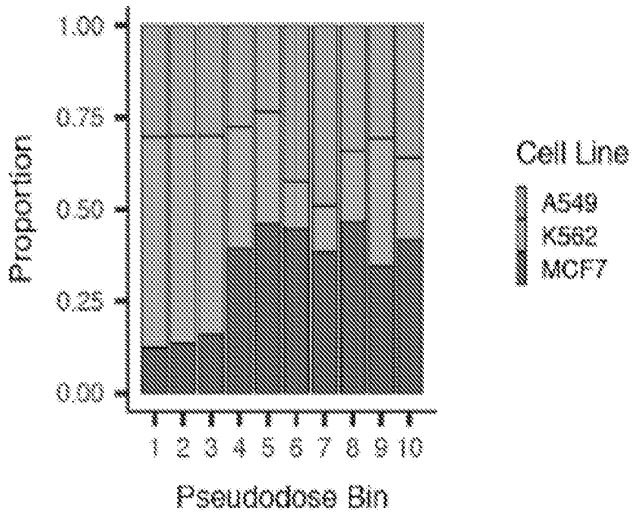


Fig. 29A

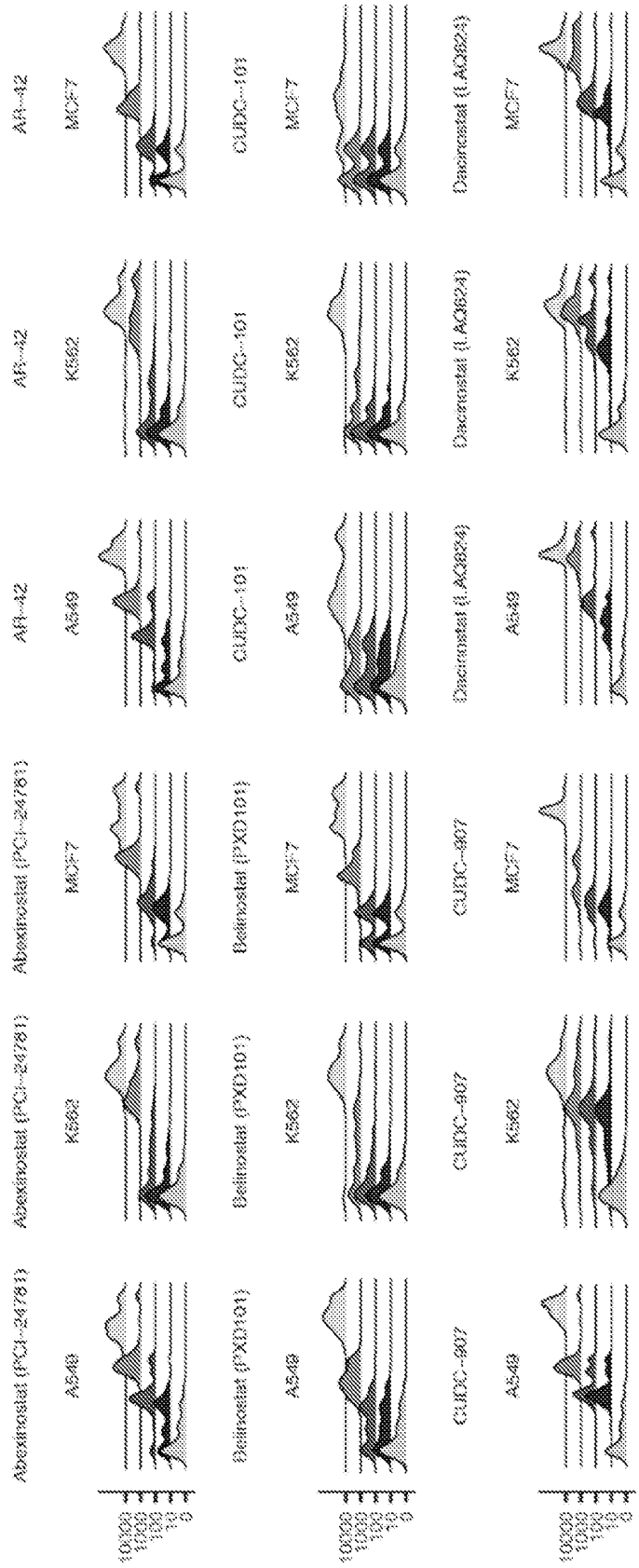

Abexinostat (PCI-24781)
A549
Abexinostat (PCI-24781)
K562
Abexinostat (PCI-24781)
MCF7
AR-42
A549
AR-42
K562
AR-42
MCF7
Belinostat (PXD101)
A549
Belinostat (PXD101)
K562
Belinostat (PXD101)
MCF7
CUDC-907
A549
CUDC-907
K562
CUDC-907
MCF7
CUDC-101
A549
CUDC-101
K562
CUDC-101
MCF7
Dacinostat (LAQ824)
A549
Dacinostat (LAQ824)
K562
Dacinostat (LAQ824)
MCF7
10000
1000
100
10
0

Fig. 29B

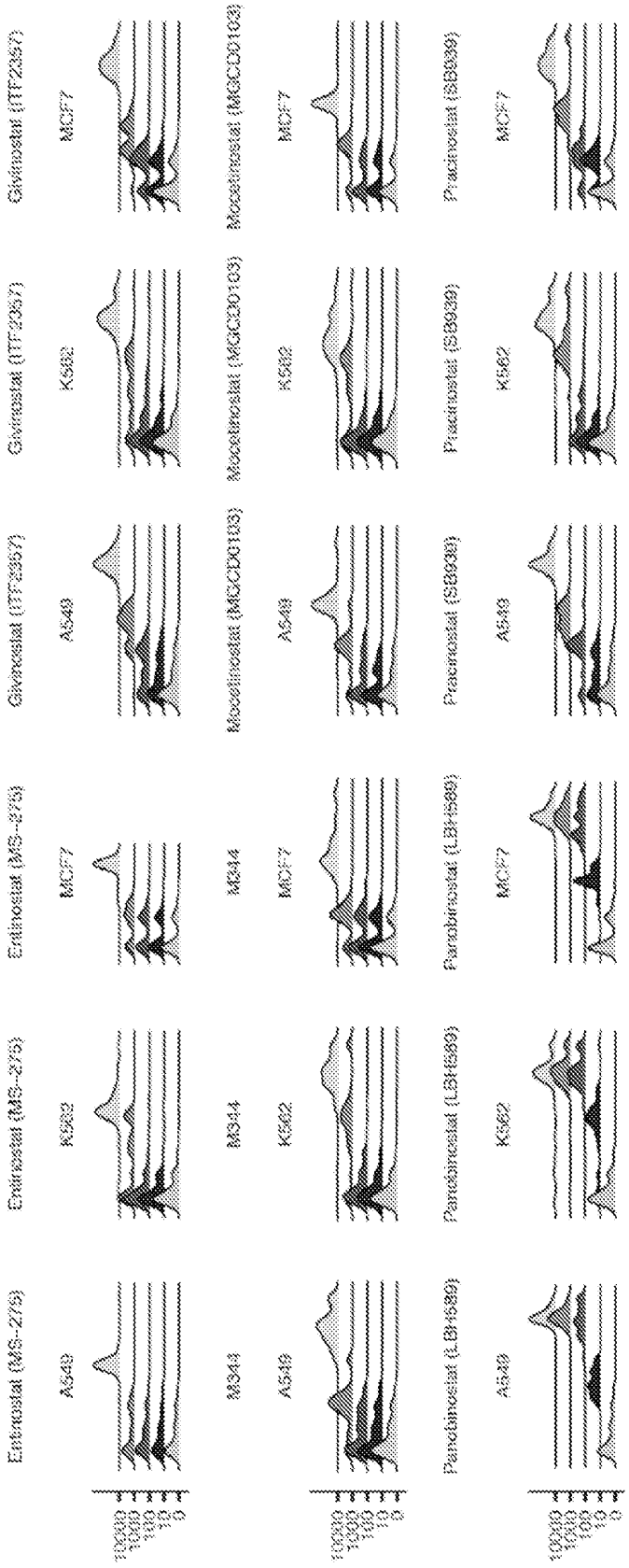

Entinostat (MS-275)
A549
Entinostat (MS-275)
K562
Entinostat (MS-275)
MCF7
Givinostat (ITF2357)
A549
Givinostat (ITF2357)
K562
Givinostat (ITF2357)
MCF7
10000
1000
100
10
0
M344
M344
M344
Mocetinostat (MGCD0103)
A549
Mocetinostat (MGCD0103)
K562
Mocetinostat (MGCD0103)
MCF7
Panobinostat (LBH589)
A549
Panobinostat (LBH589)
K562
Panobinostat (LBH589)
MCF7
Pracinostat (SB939)
A549
Pracinostat (SB939)
K562
Pracinostat (SB939)
MCF7

Fig. 29C

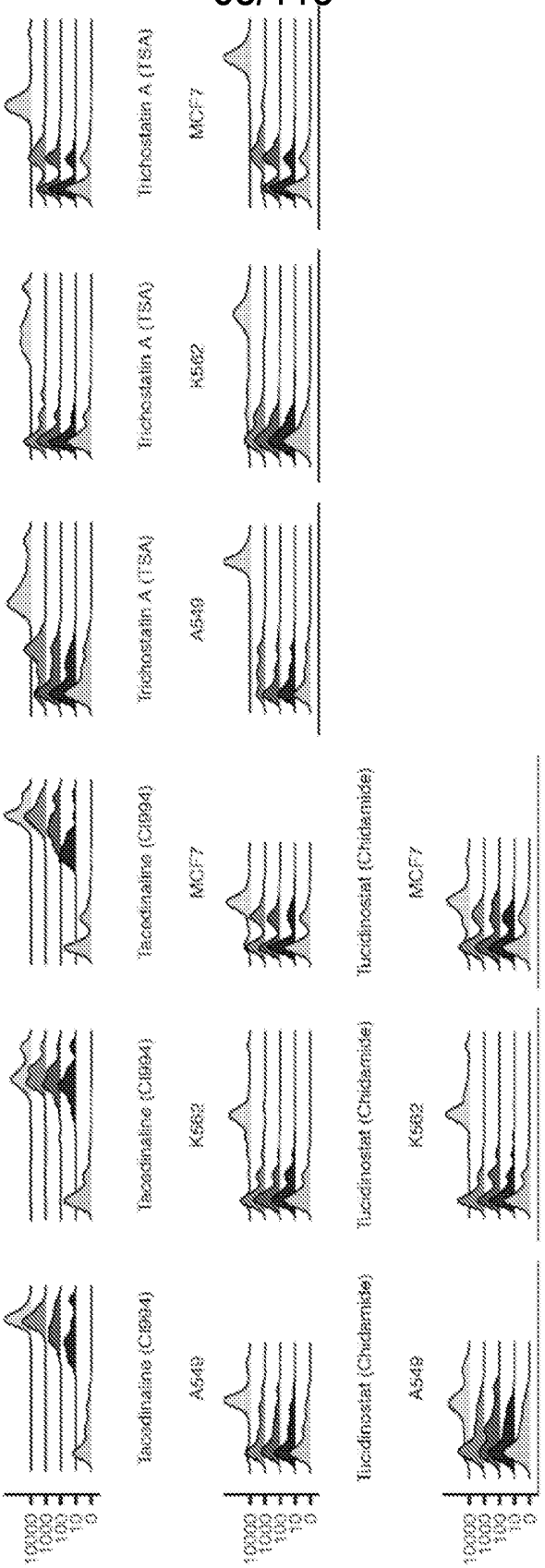

Panobinostat
MCF7
Trichostatin A (TSA)
MCF7
Panobinostat
K562
Trichostatin A (TSA)
K562
Panobinostat
A549
Trichostatin A (TSA)
A549
sinostat (JNJ-26481585) 2I
MCF7
Tacedinaline (CI994)
MCF7
sinostat (JNJ-26481585) 2I
K562
Tacedinaline (CI994)
K562
sinostat (JNJ-26481585) 2I
A549
Tacedinaline (CI994)
A549
Tucidinostat (Chidamide)
MCF7
Tucidinostat (Chidamide)
K562
Tucidinostat (Chidamide)
A549
10000
1000
100
10
0

Fig. 30A

A

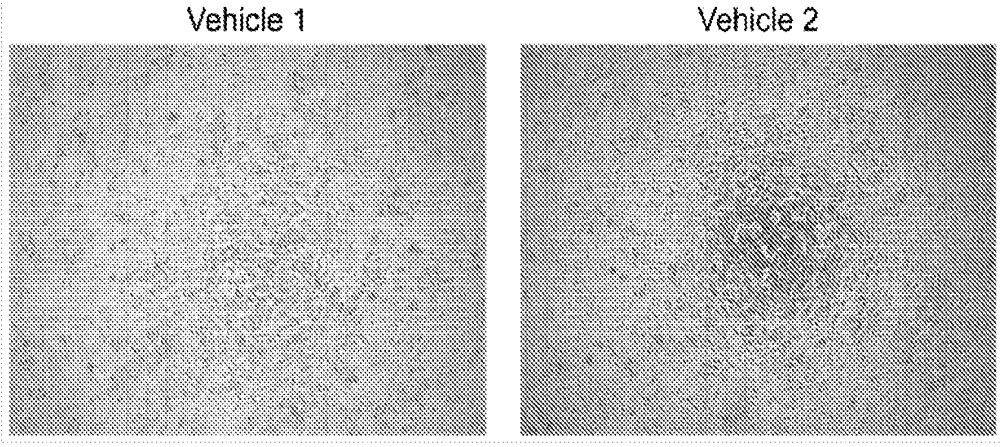


Fig. 30B

B

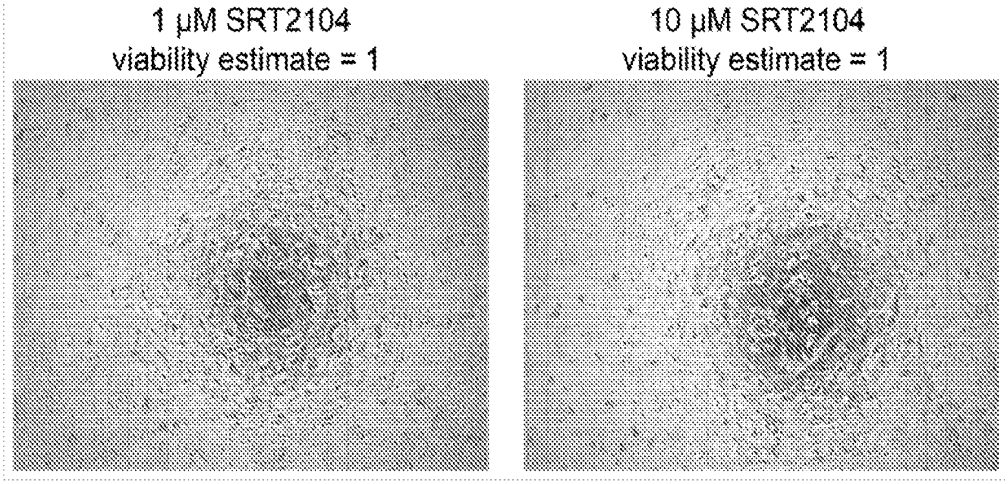


Fig. 30C

C

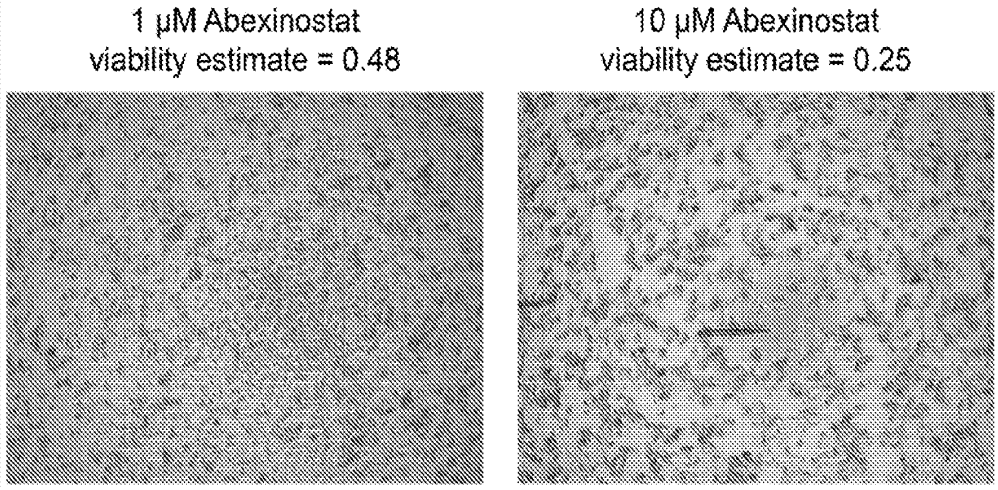


Fig. 31A

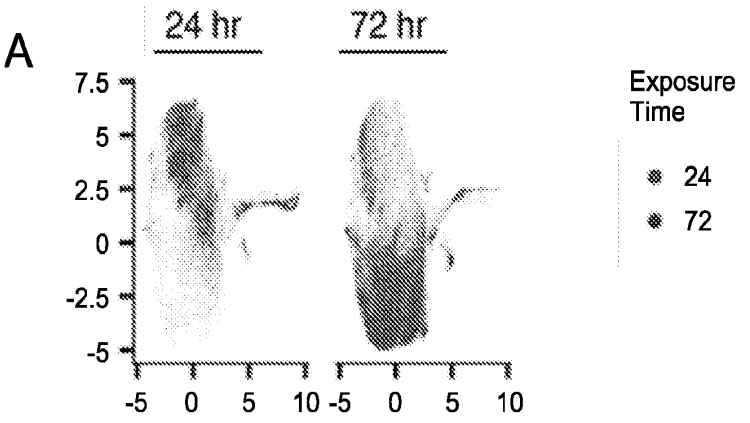


Fig. 31B

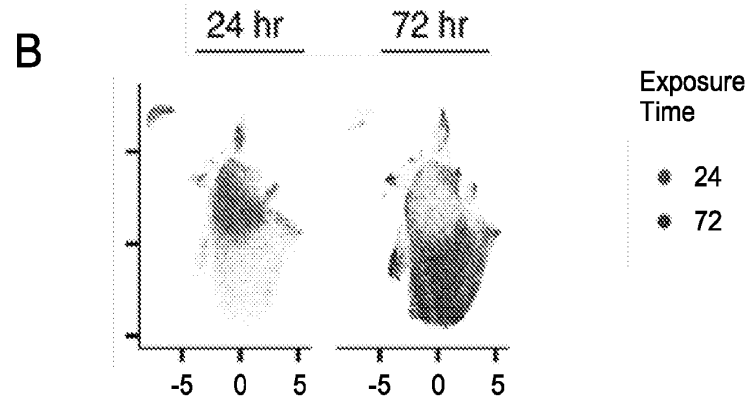


Fig. 31C

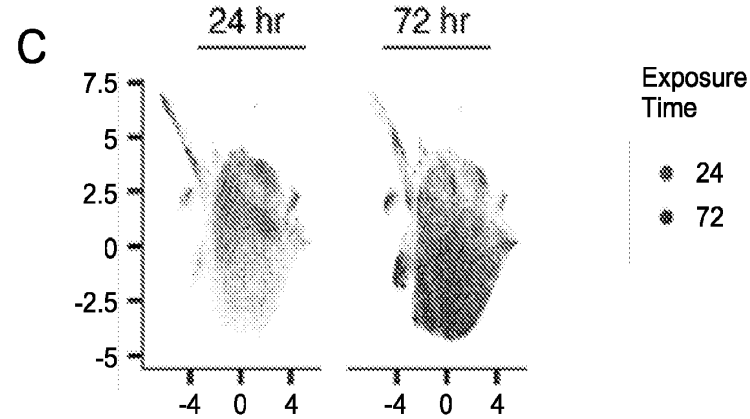


Fig. 31D

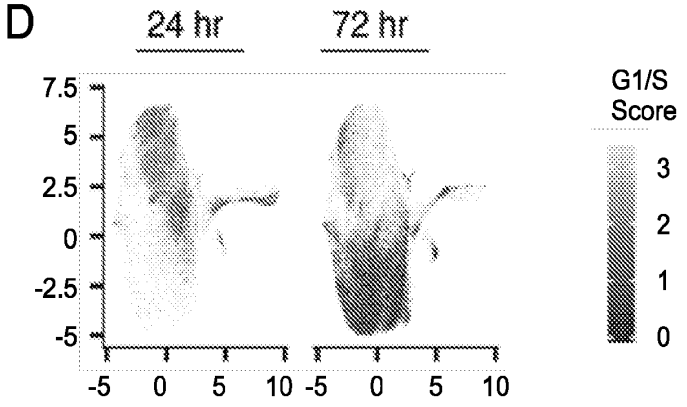


Fig. 31E

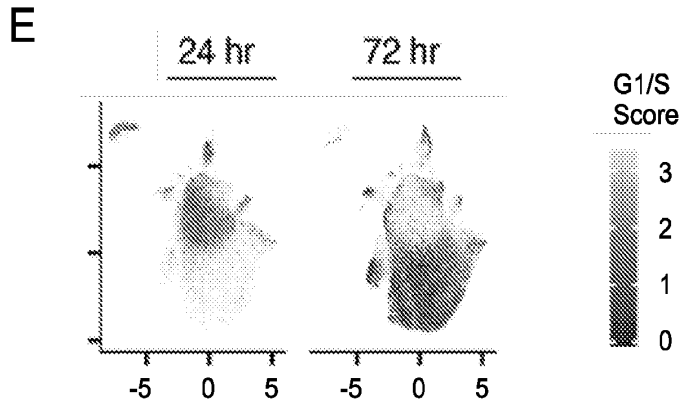


Fig. 31F

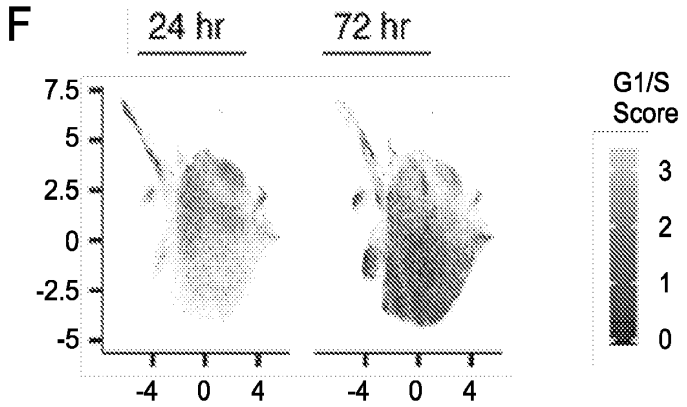


Fig. 31G

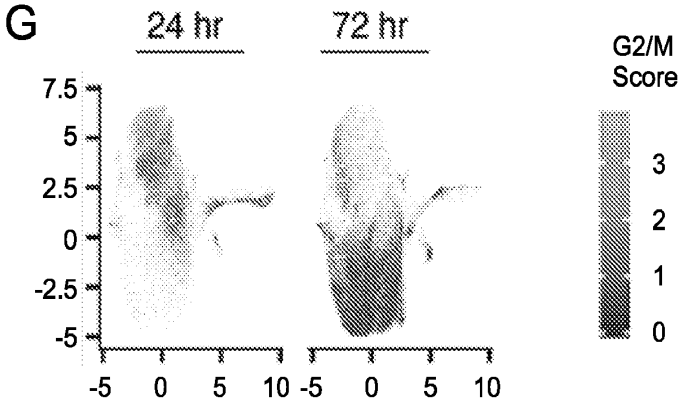


Fig. 31H

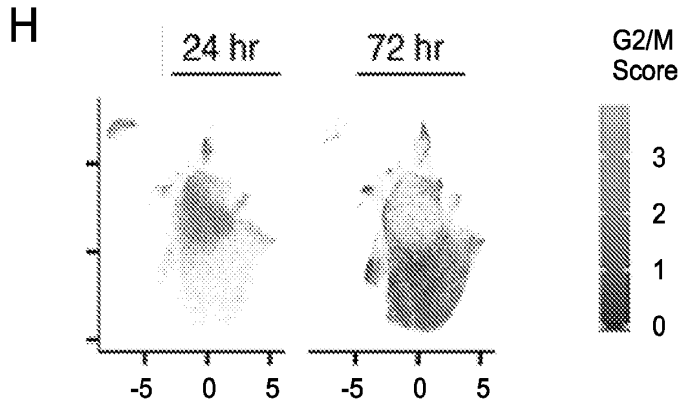


Fig. 31I

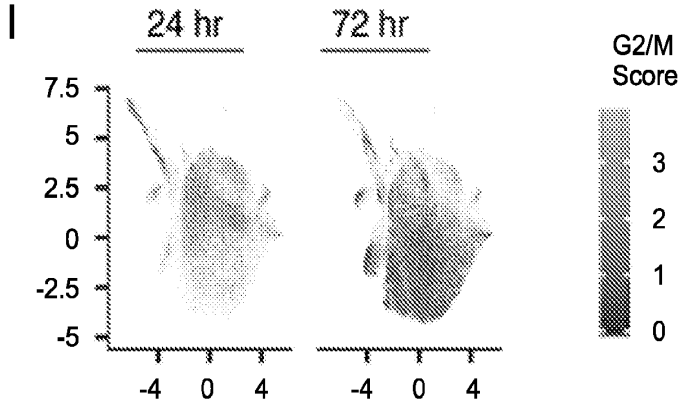


Fig. 31J

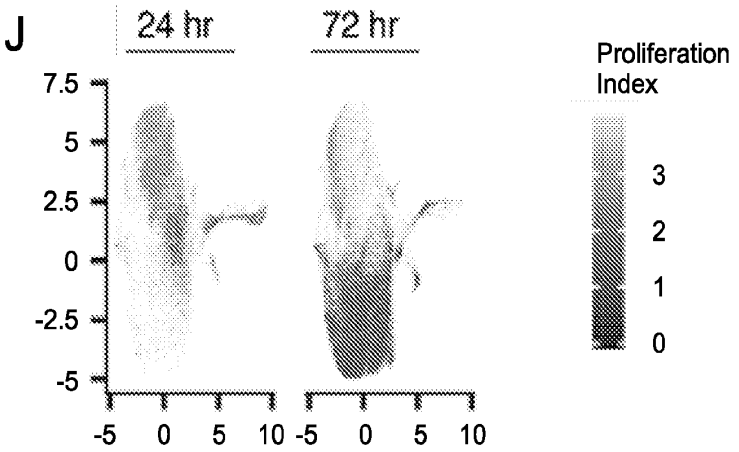


Fig. 31K

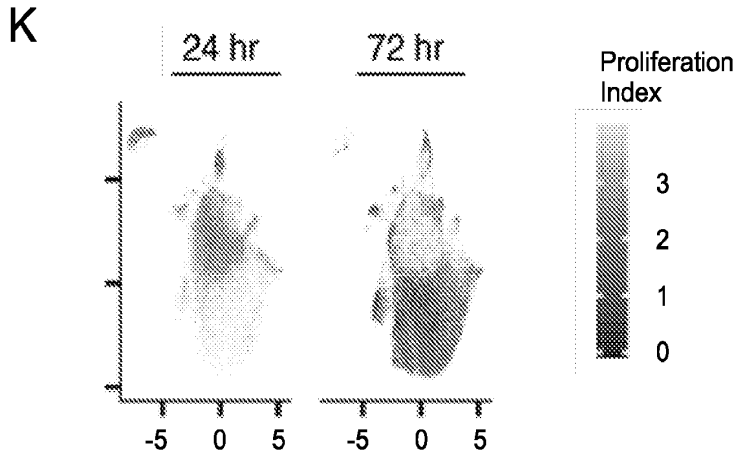


Fig. 31L

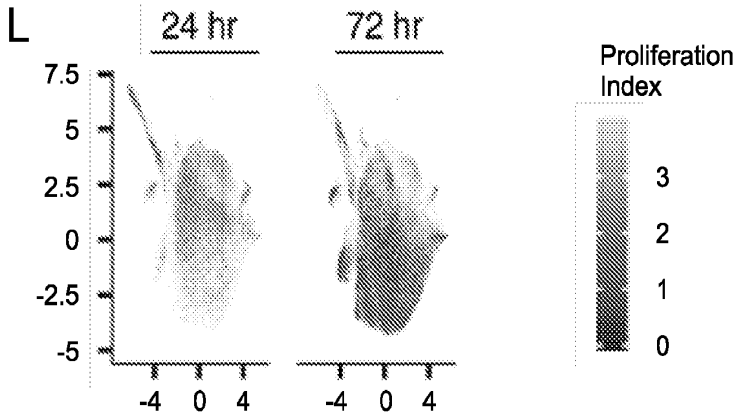


Fig. 31M

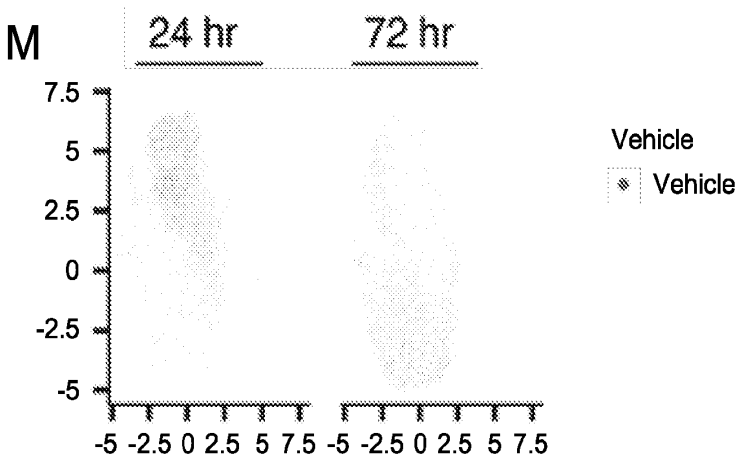


Fig. 31N

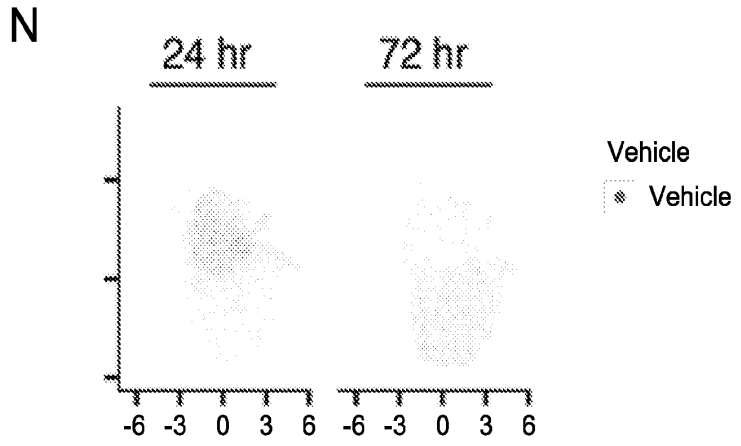


Fig. 31O

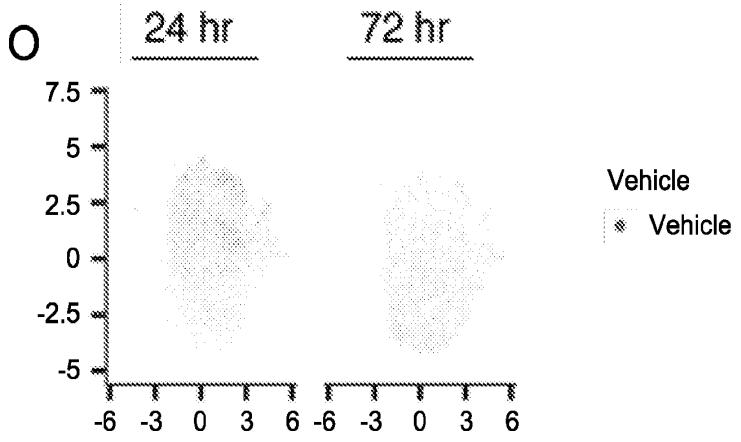


Fig. 31P

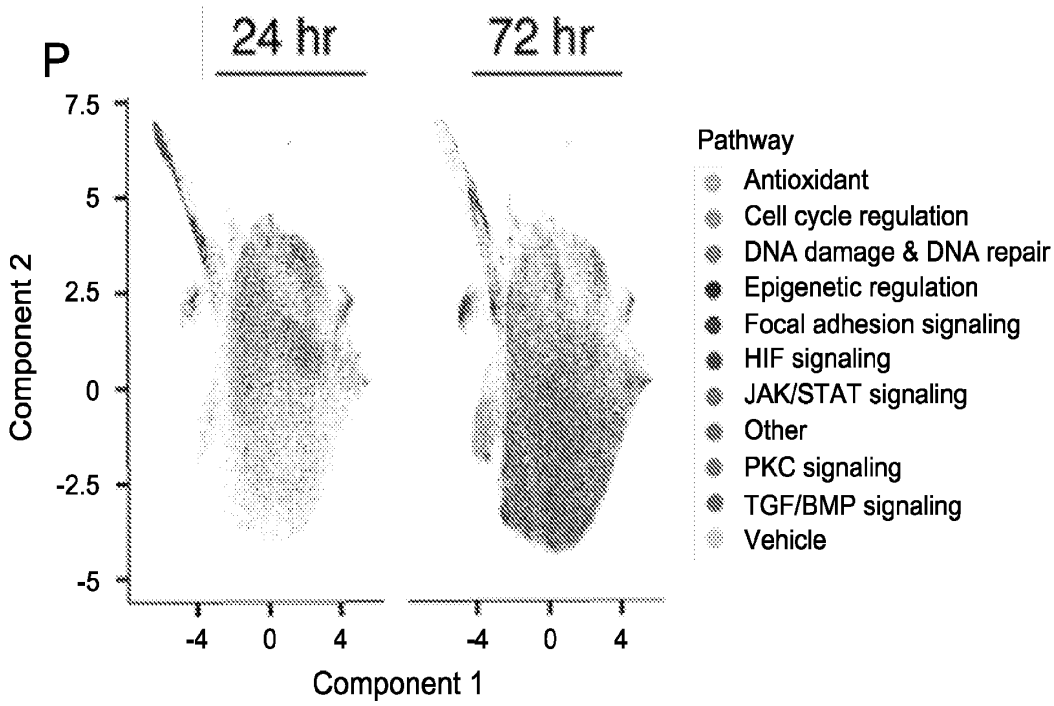


Fig. 31Q

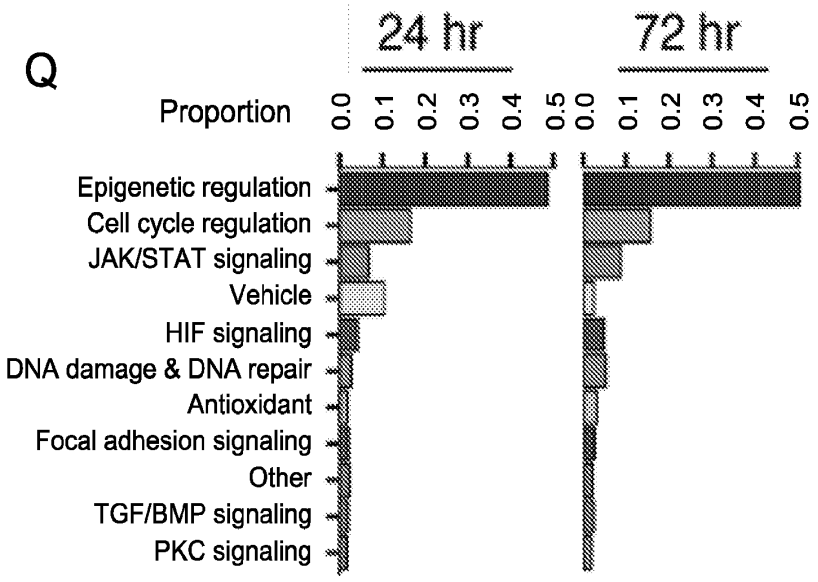


Fig. 31R

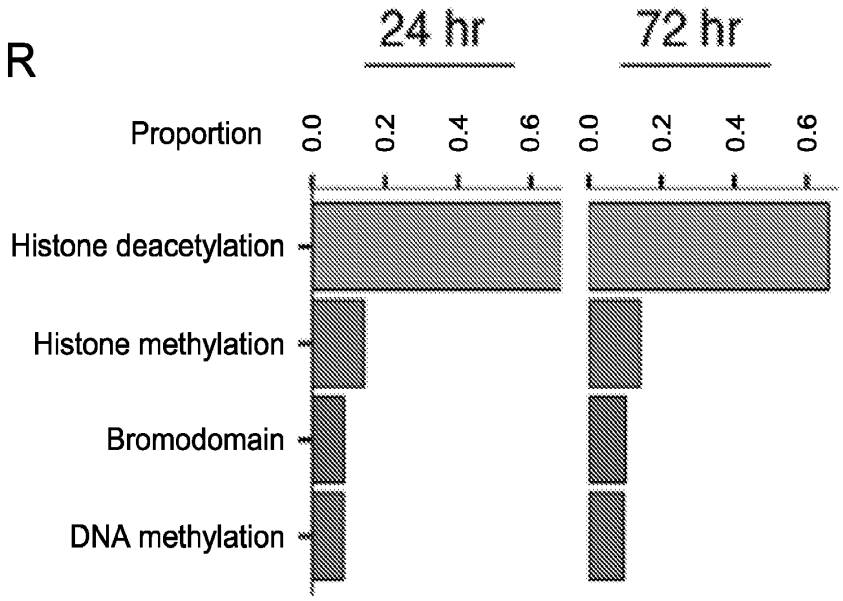


Fig. 31S

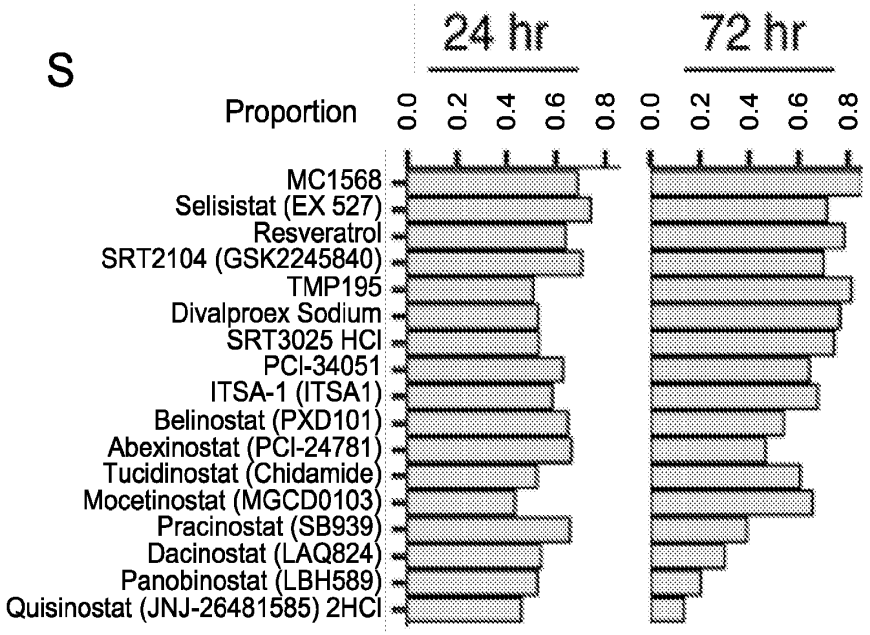


Fig. 32A

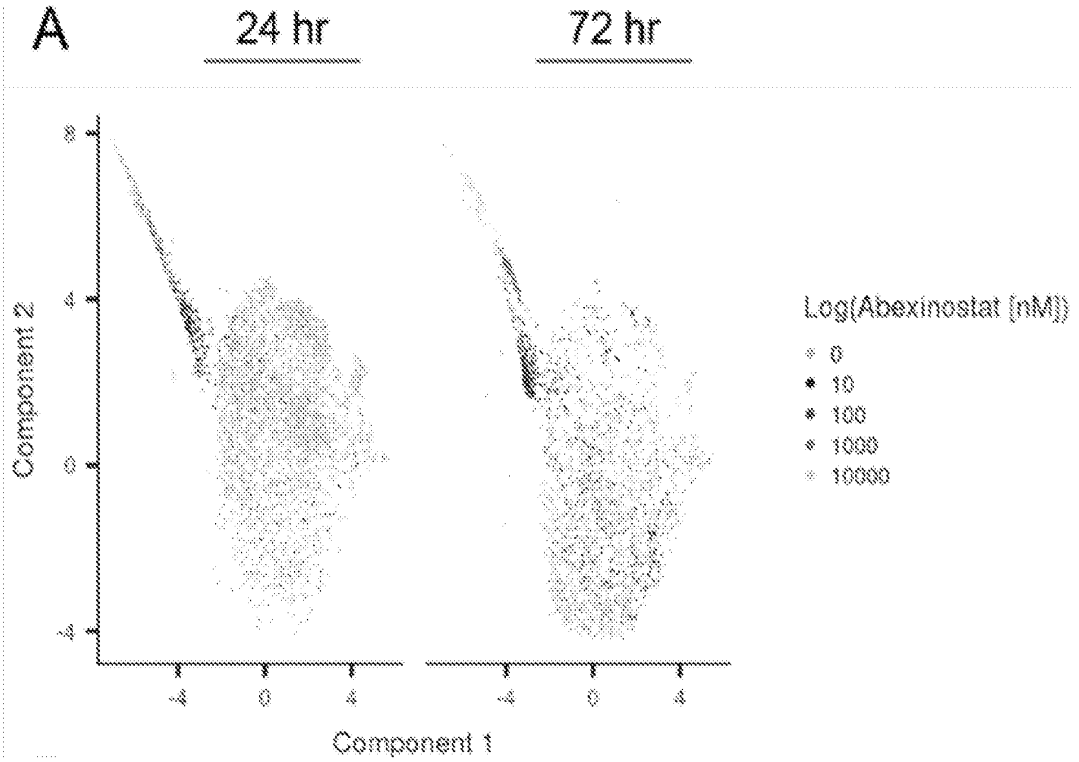


Fig. 32B

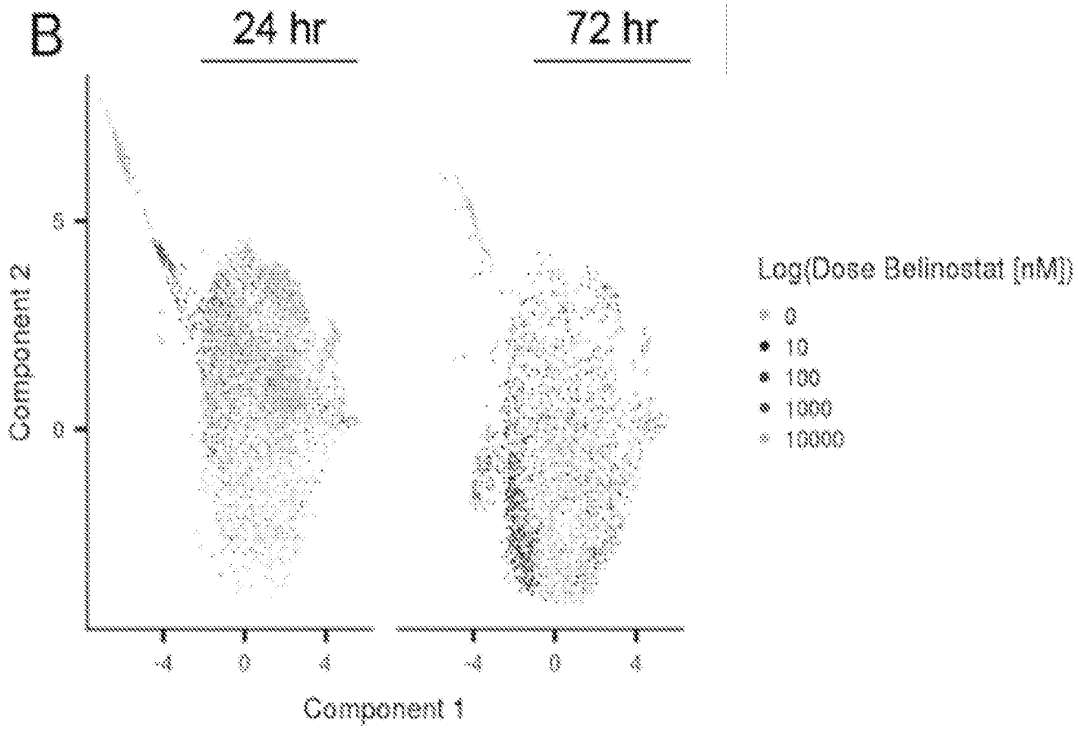


Fig. 32C

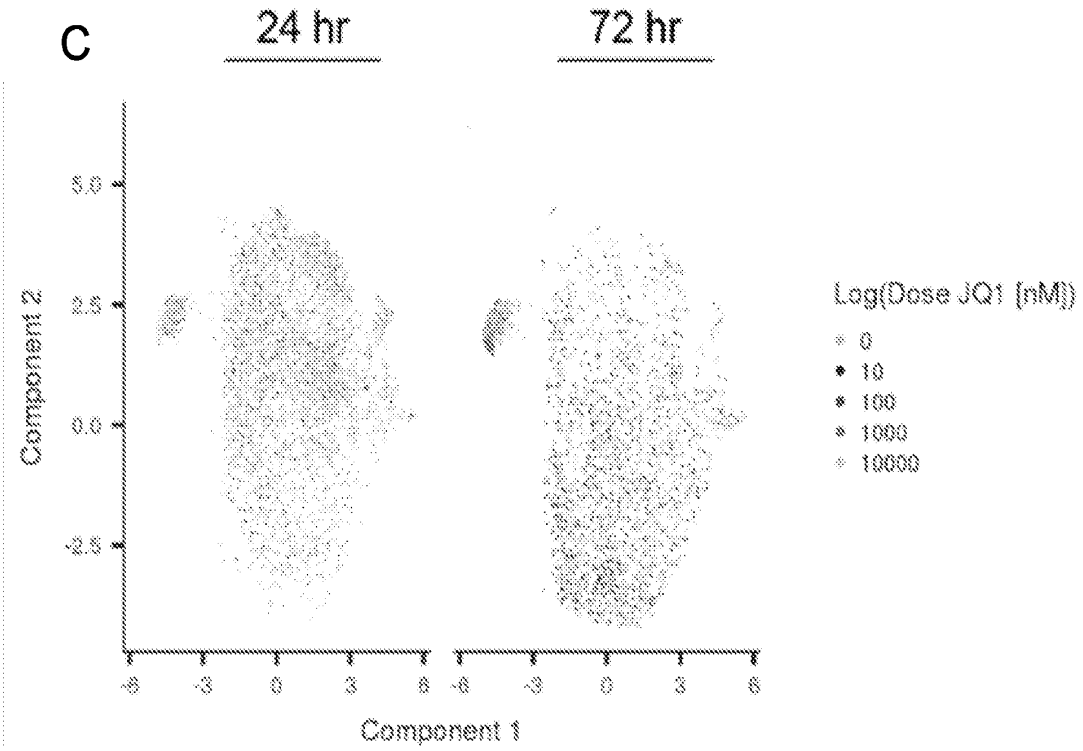
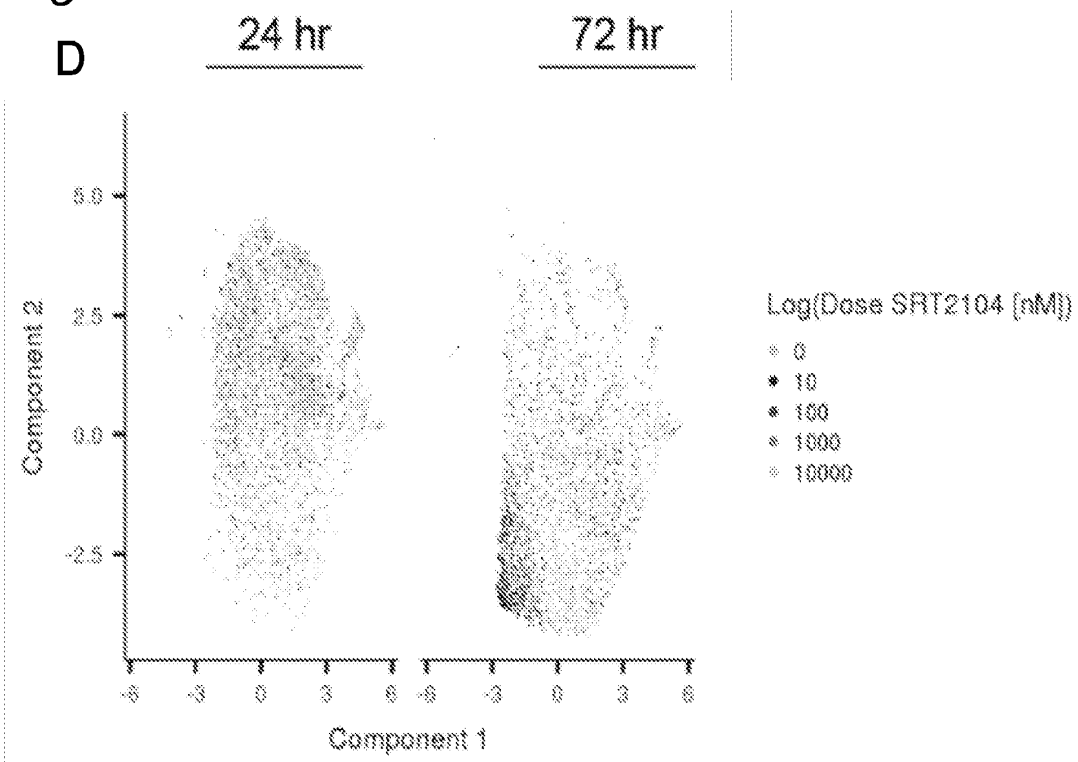


Fig. 32D



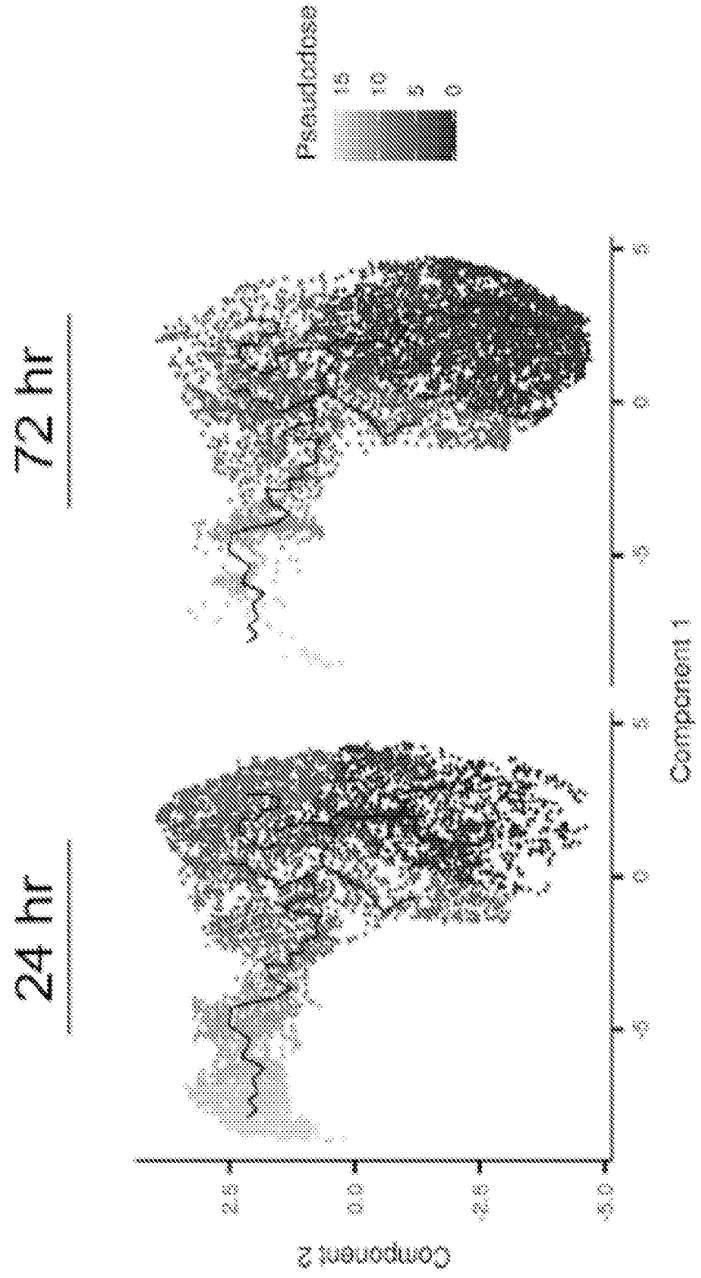
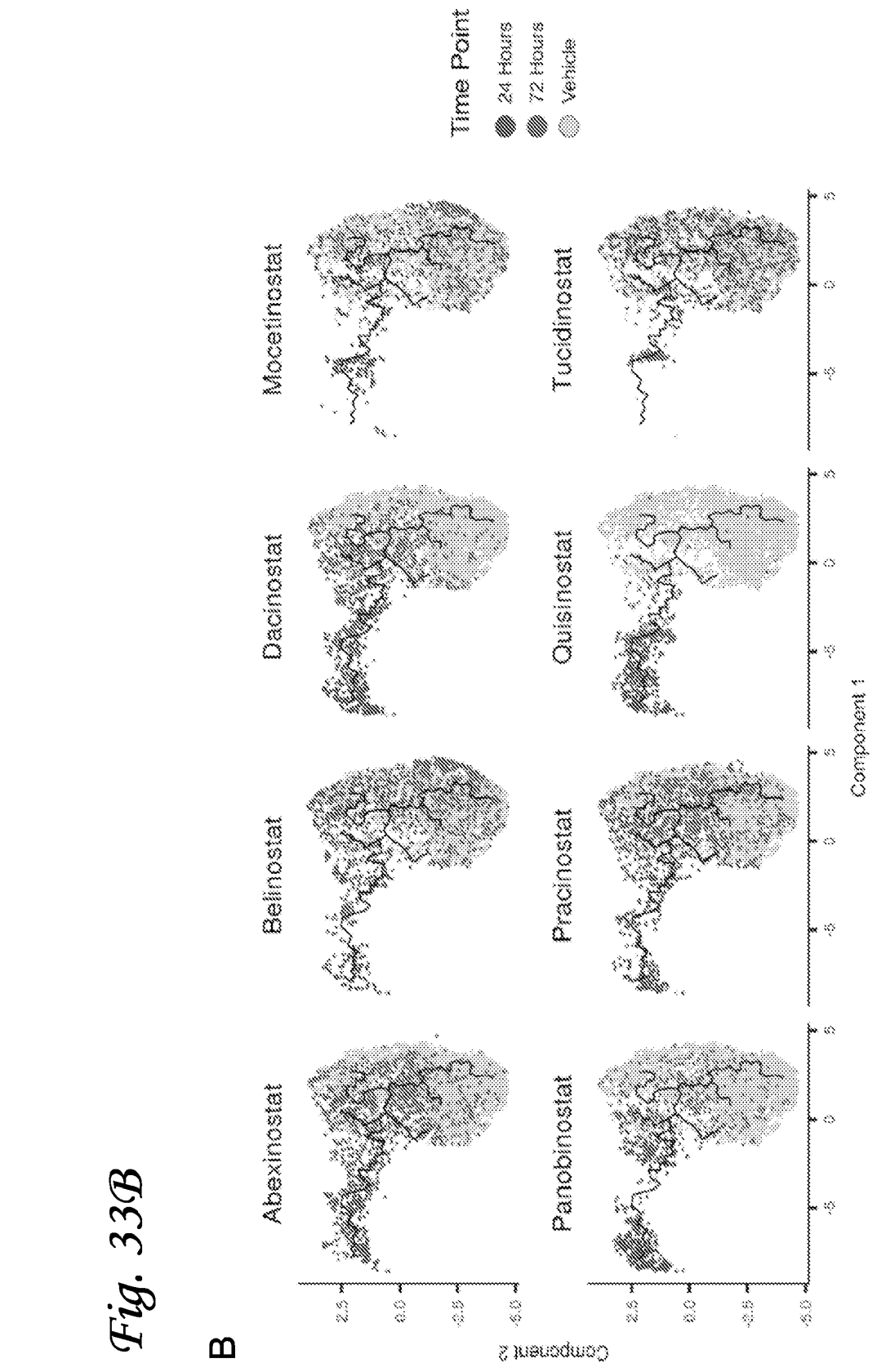
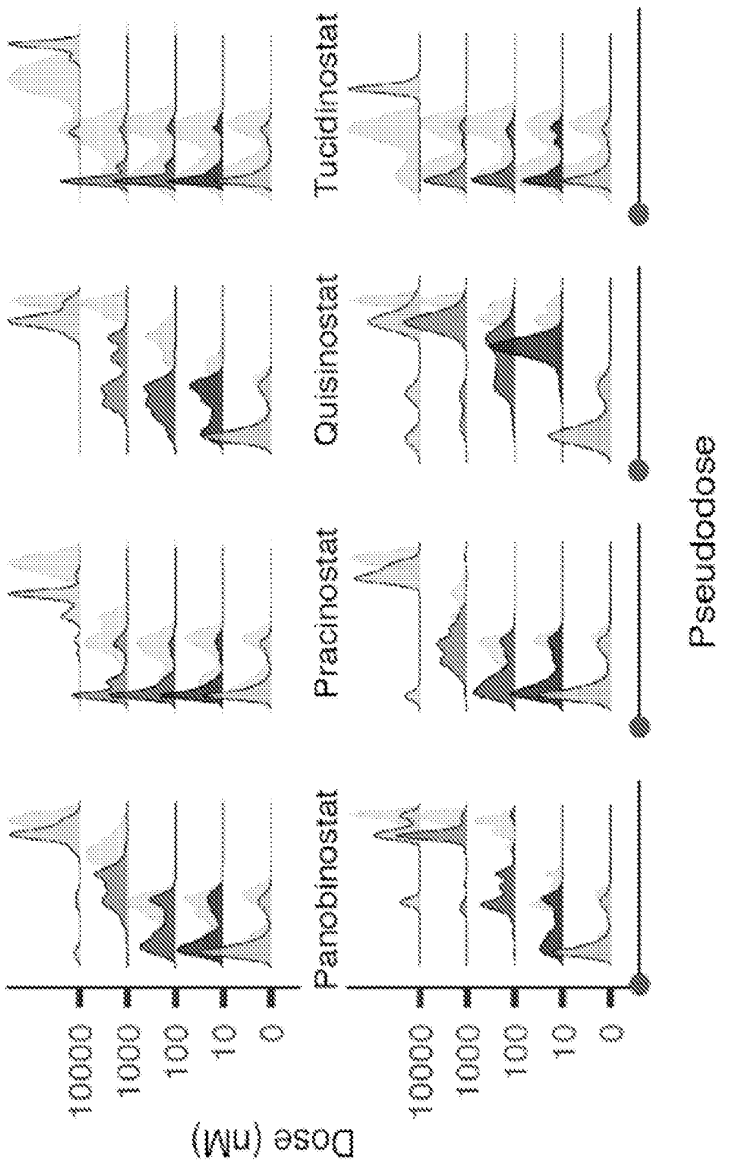

A
24 hr
72 hr
Pseudotase
15
10
5
0
Component 1
Component 2
2.5
0.0
-2.5
-5.0
5
0
-5

Fig. 33A

Fig. 33B

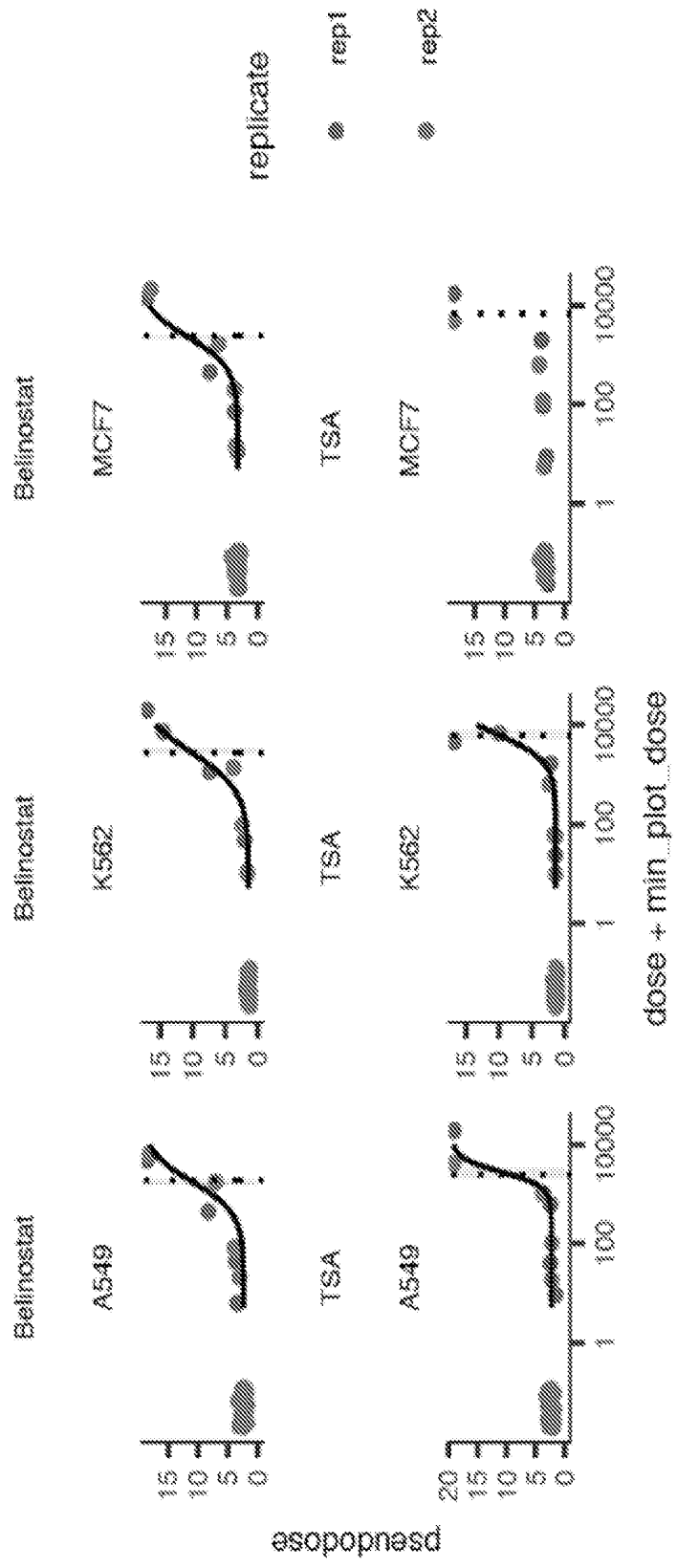

B
Time Point
24 Hours
72 Hours
Vehicle
Abexinostat
Belinostat
Dacinostat
Mocetinostat
Panobinostat
Pracinostat
Quisinostat
Tucidinostat
Component 2
Component 1


Abexinostat
Belinostat
Dacinostat
Mocetinostat
Panobinostat
Pracinostat
Quisinostat
Tucidinostat
Dose (nM)
10000
1000
100
10
0
Pseudodose

C

Fig. 33C

Fig. 34A


A
Belinostat
TSA
A549
K562
MCF7
pseudodose
dose + min_plot_dose
replicate
rep1
rep2

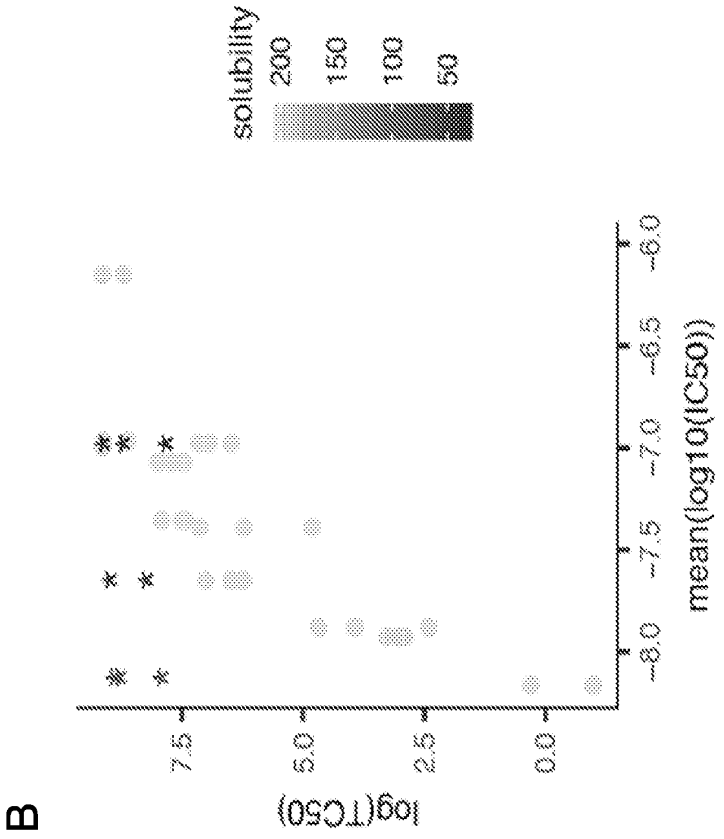

B
solubility
200
150
100
50
log(TC50)
7.5
5.0
2.5
0.0
mean(log10(IC50))
−8.0
−7.5
−7.0
−6.5
−6.0

Fig. 34B

Fig. 34C

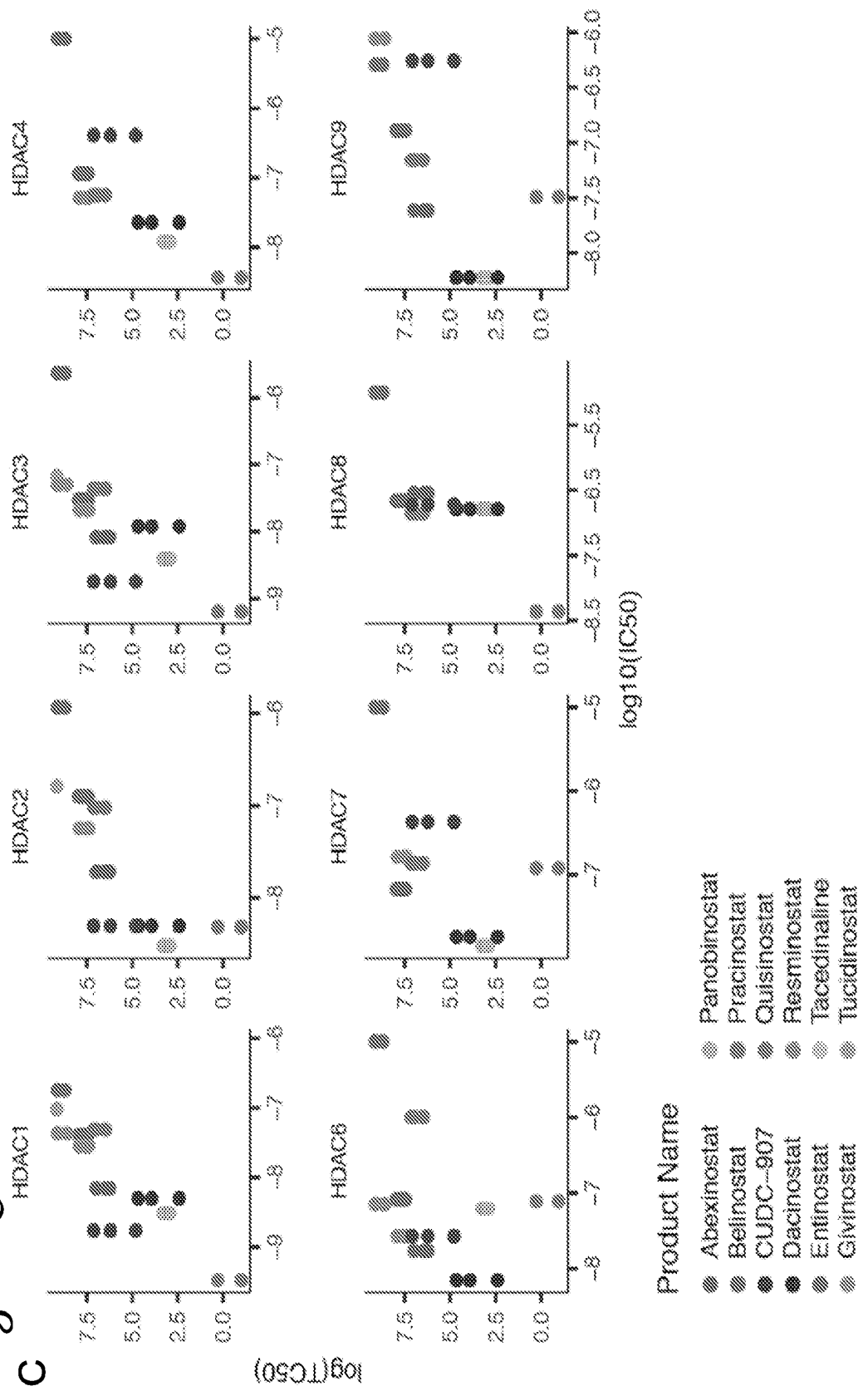

HDAC1
HDAC2
HDAC3
HDAC4
HDAC6
HDAC7
HDAC8
HDAC9
log(TC50)
log10(IC50)
Product Name
Abexinostat
Belinostat
CUDC−907
Dacinostat
Entinostat
Givinostat
Panobinostat
Pracinostat
Quisinostat
Resminostat
Tacedinaline
Tucidinostat

Fig. 35A

A

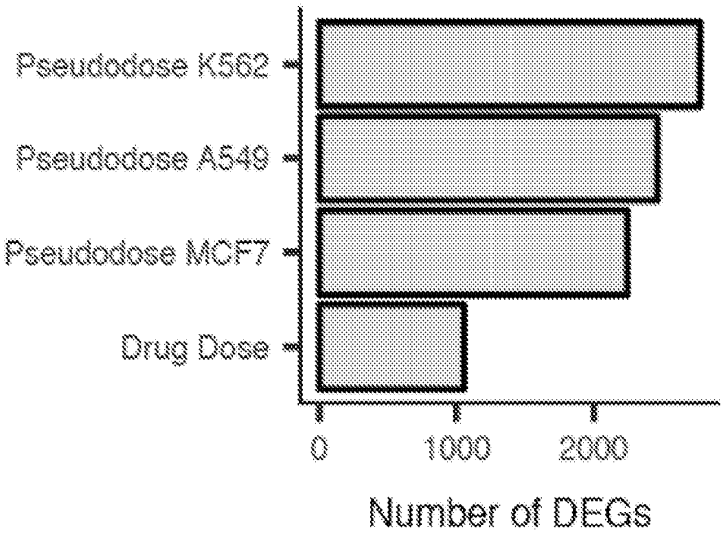
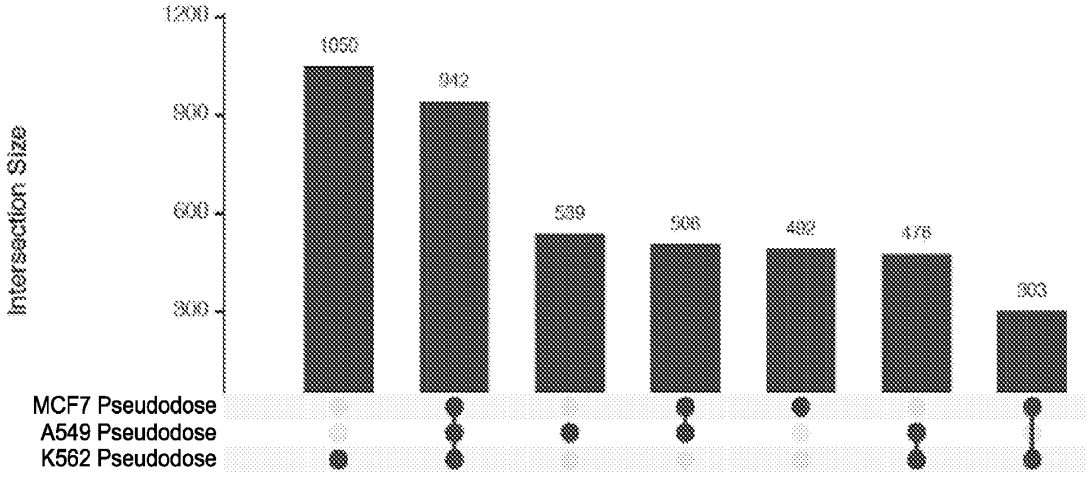


Fig. 35B

B



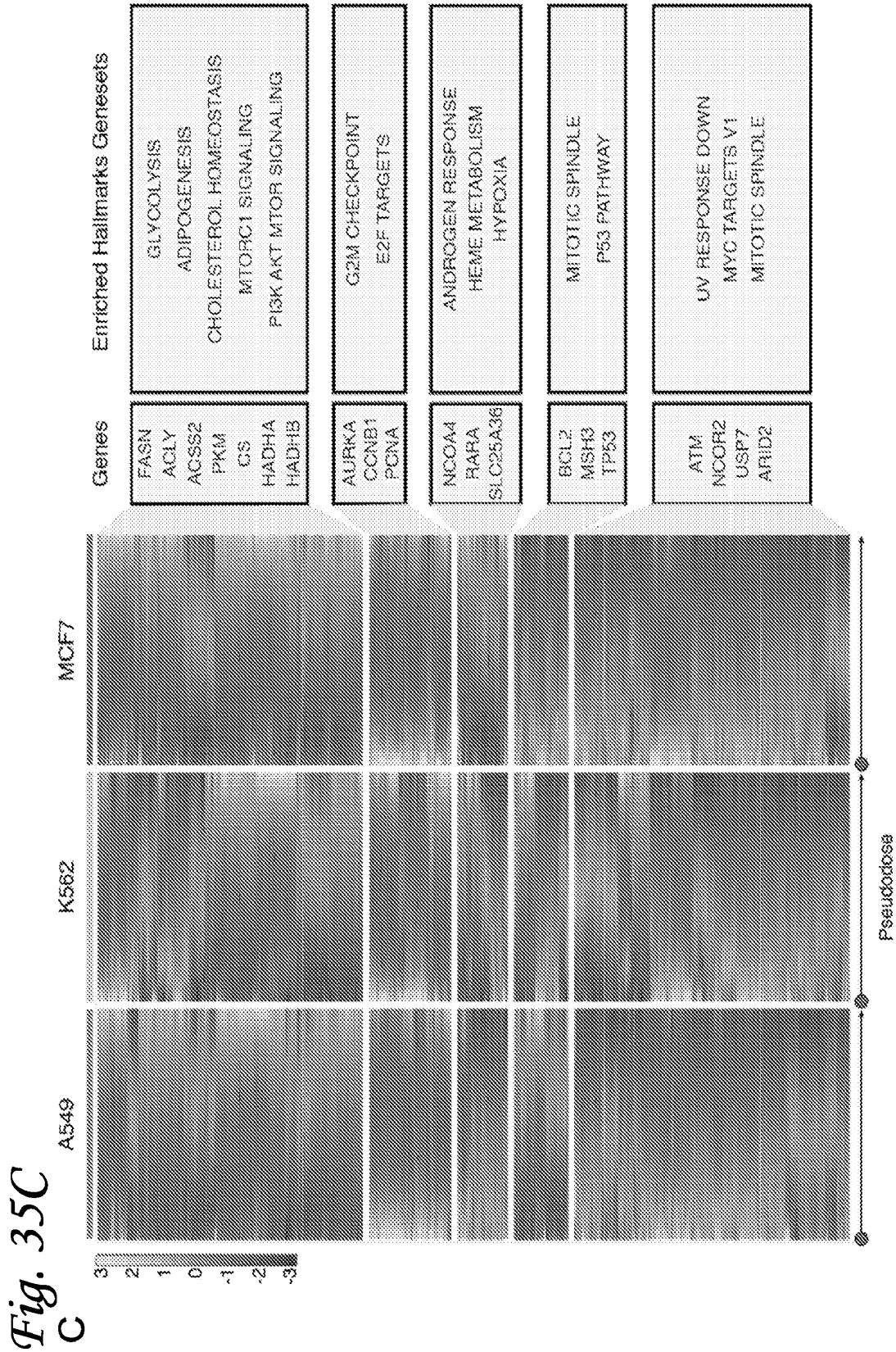

Enriched Hallmarks Genesets
Genes
GLYCOLYSIS
ADIPOGENESIS
CHOLESTEROL HOMEOSTASIS
MTORC1 SIGNALING
PI3K AKT MTOR SIGNALING
FASN
ACLY
ACSS2
PKM
CS
HADHA
HADHB
G2M CHECKPOINT
E2F TARGETS
AURKA
CCNB1
PCNA
ANDROGEN RESPONSE
HEME METABOLISM
HYPOXIA
NCOA4
RARA
SLC25A36
MITOTIC SPINDLE
P53 PATHWAY
BCL2
MSH3
TP53
UV RESPONSE DOWN
MYC TARGETS V1
MITOTIC SPINDLE
ATM
NCOR2
USP7
ARID2
A549
K562
MCF7
3
2
1
0
-1
-2
-3
Pseudodose

Fig. 35C

Fig. 36A

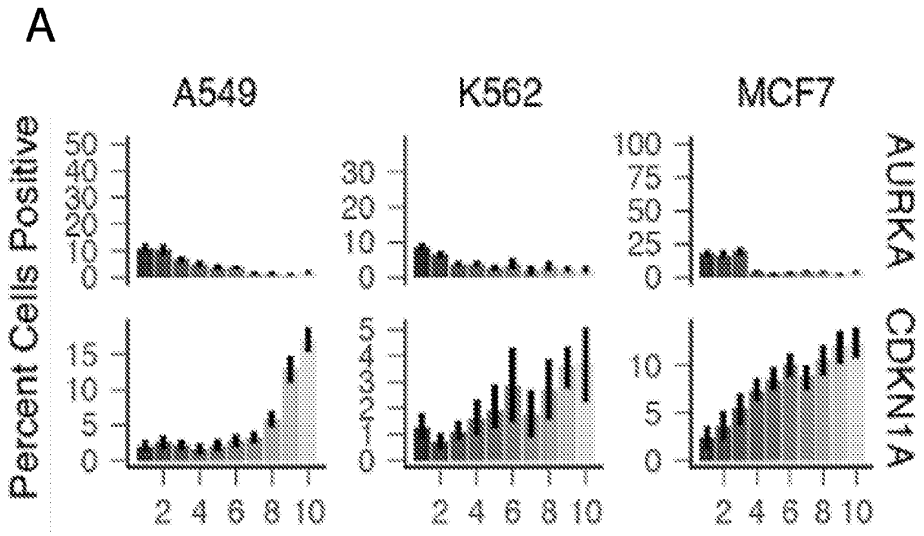
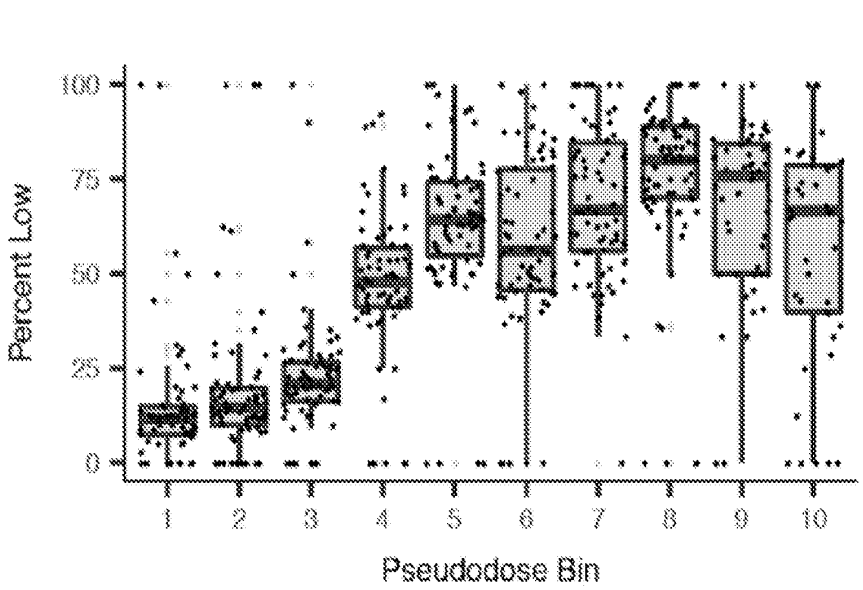


Fig. 36B



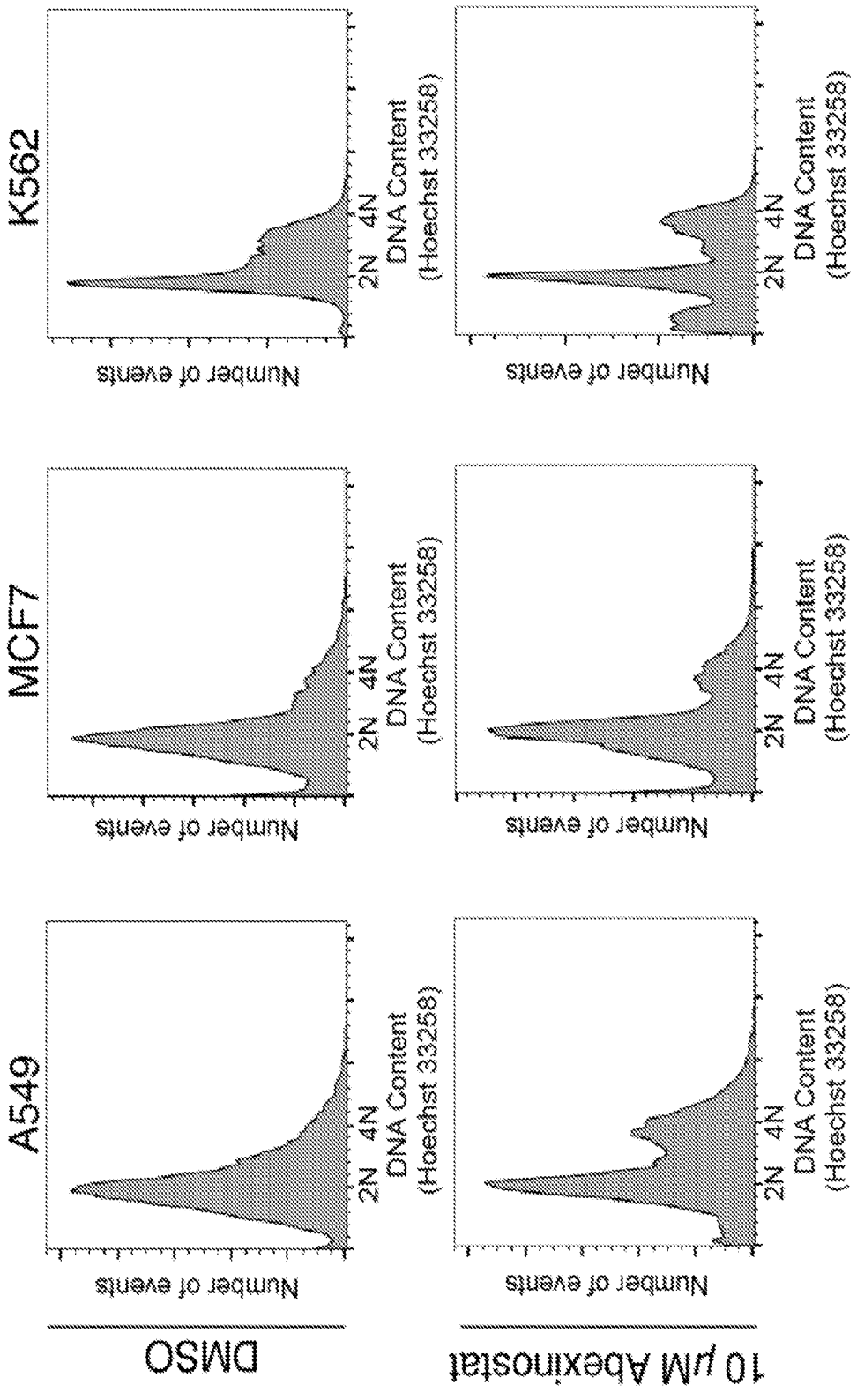
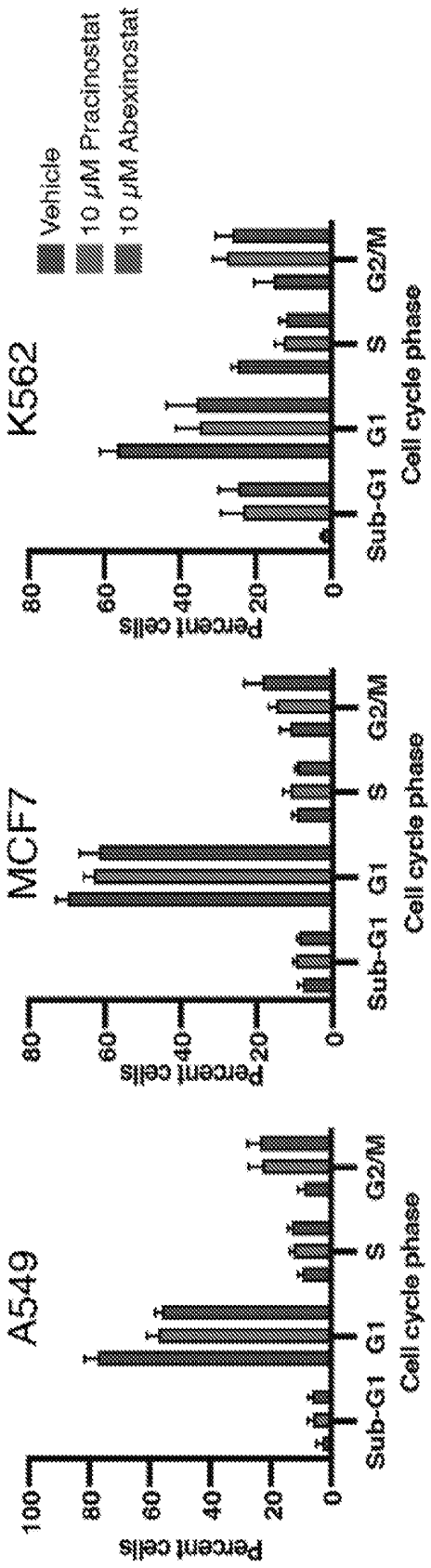

A549
MCF7
K562
DMSO
10 μM Abexinostat
Number of events
2N 4N
DNA Content
(Hoechst 33258)

Fig. 36C
C

Fig. 36D

D


A549
MCF7
K562
Vehicle
10 μM Pracinostat
10 μM Abeximostat
Percent cells
0
20
40
60
80
100
Sub-G1
G1
S
G2/M
Cell cycle phase

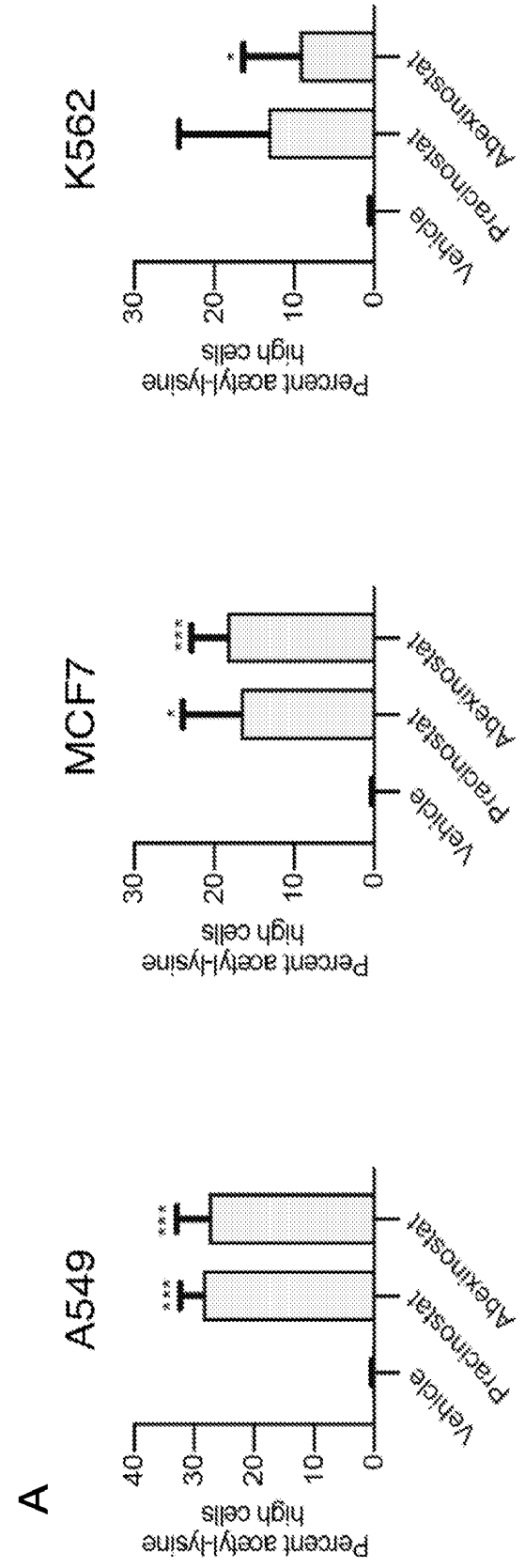

A
A549
K562
MCF7
Percent acetyl-lysine high cells
0
10
20
30
40
Vehicle
Pracinostat
Abexinostat
***
*

Fig. 37A

Fig. 37B

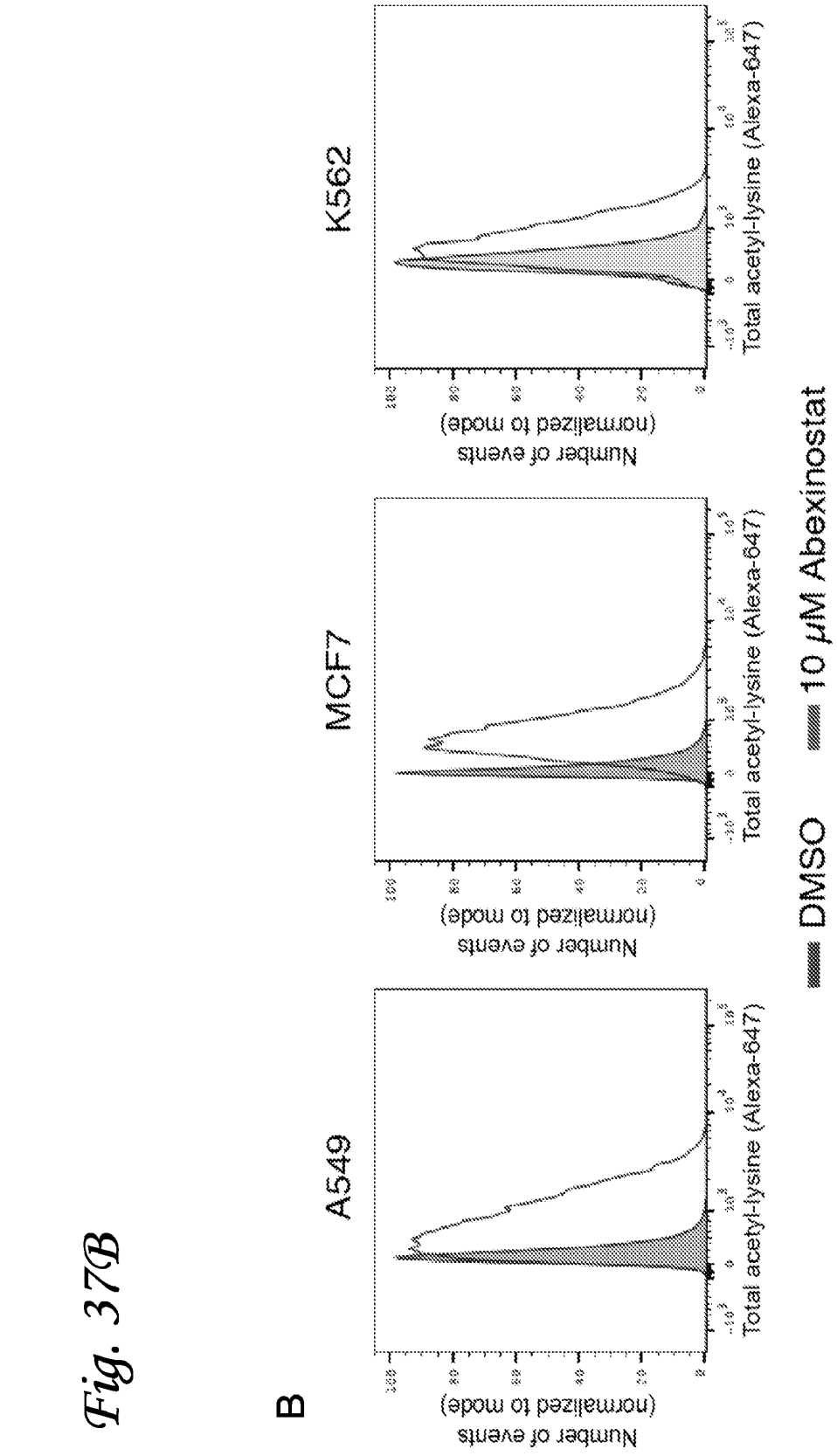

B
A549
MCF7
K562
Number of events (normalized to mode)
Total acetyl-lysine (Alexa-647)
DMSO
10 μM Abexinostat

Fig. 38A

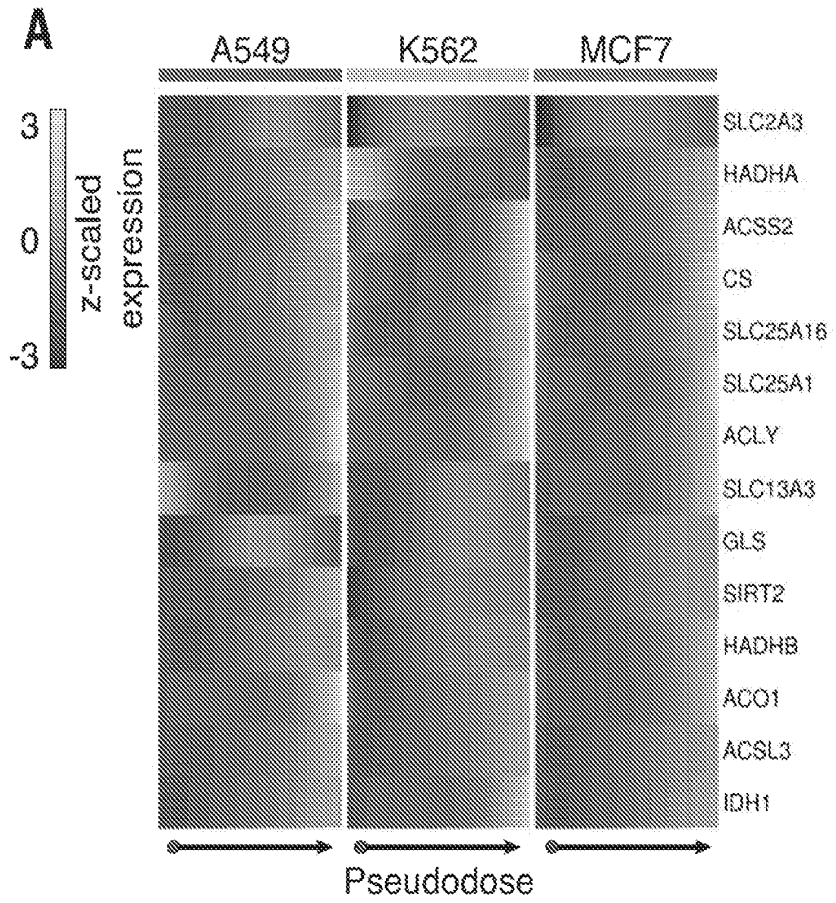
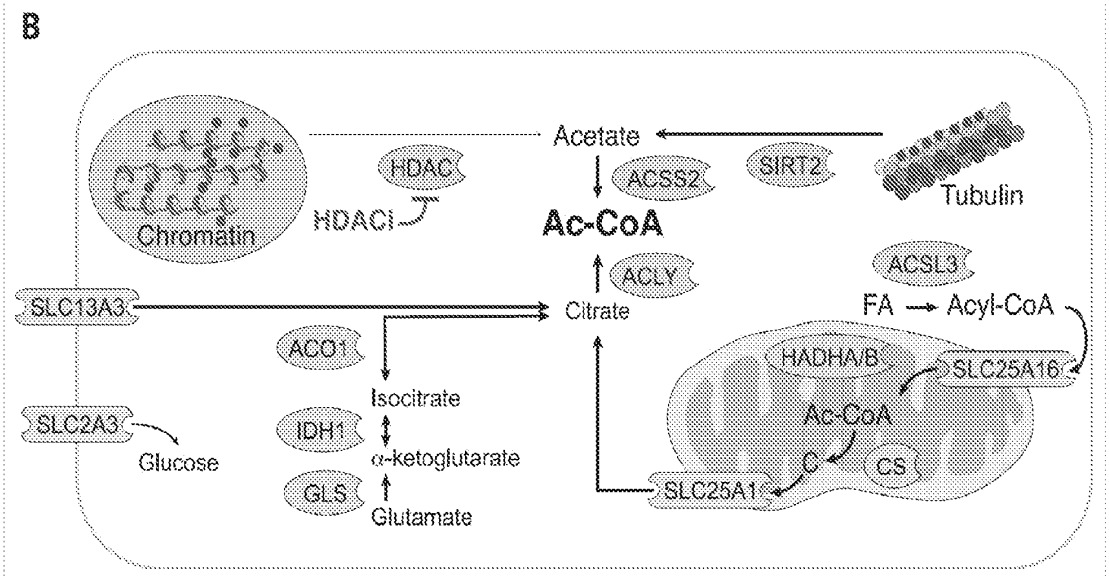


Fig. 38B



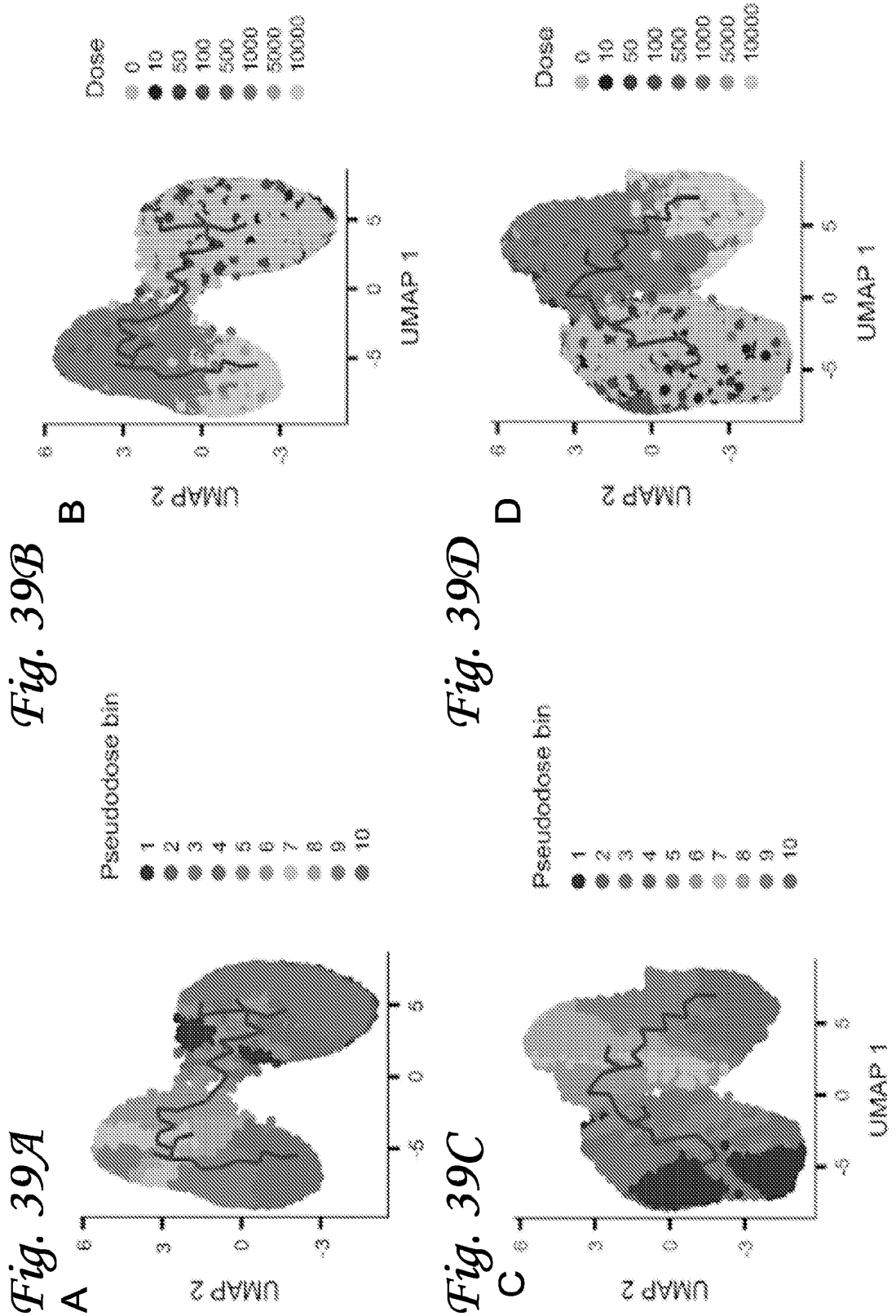


Fig. 39A

Fig. 39B

Fig. 39C

Fig. 39D

Fig. 39E

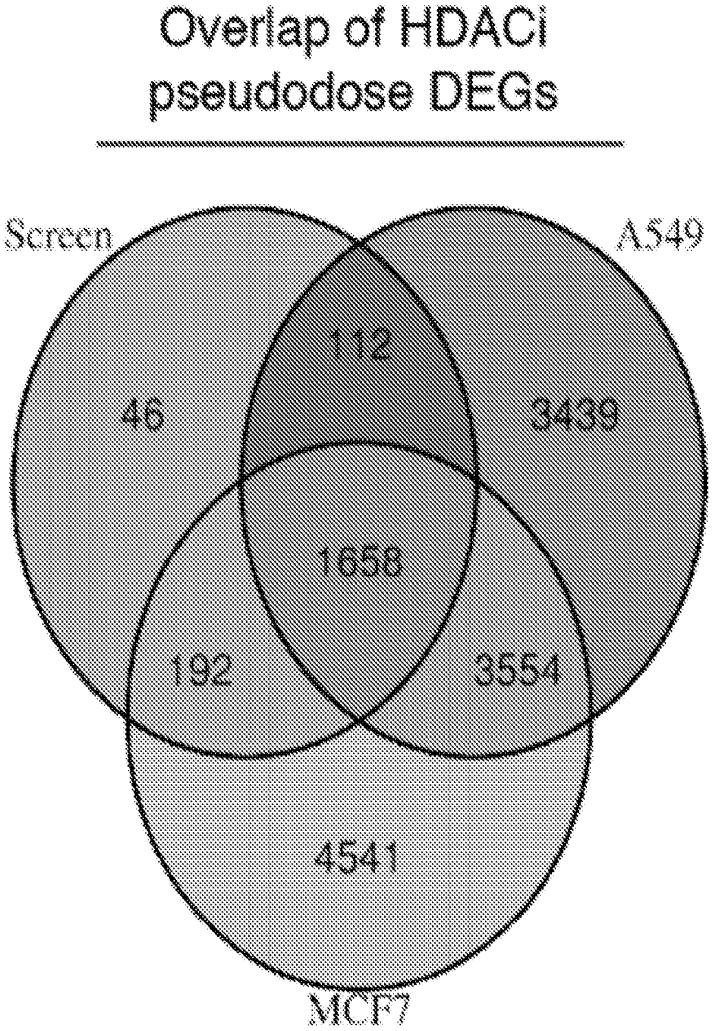

Overlap of HDACi pseudodose DEGs
Screen
A549
MCF7
46
112
3439
1658
192
3554
4541

Fig. 39F

F

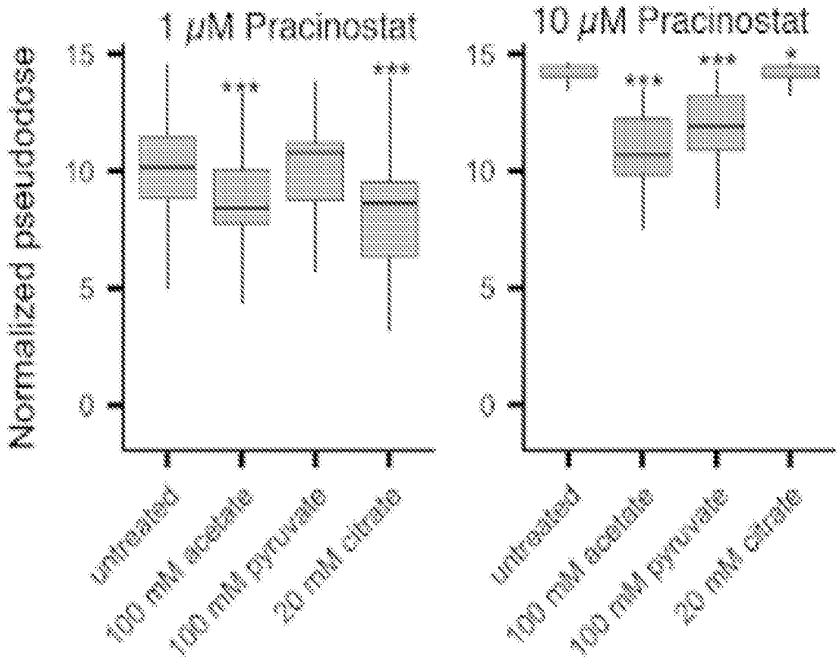


Fig. 39G

G

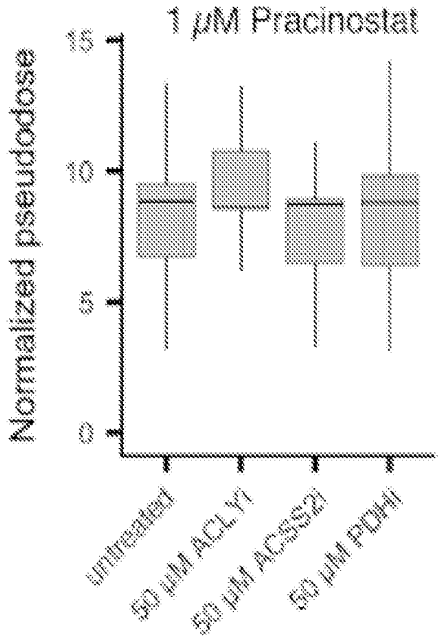


Fig. 39H

H

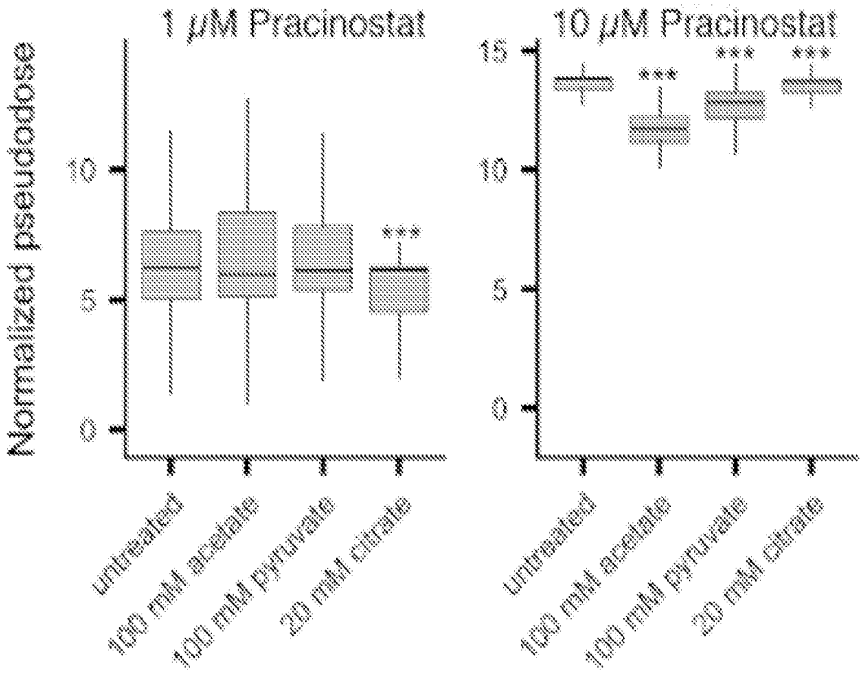


Fig. 39I

I

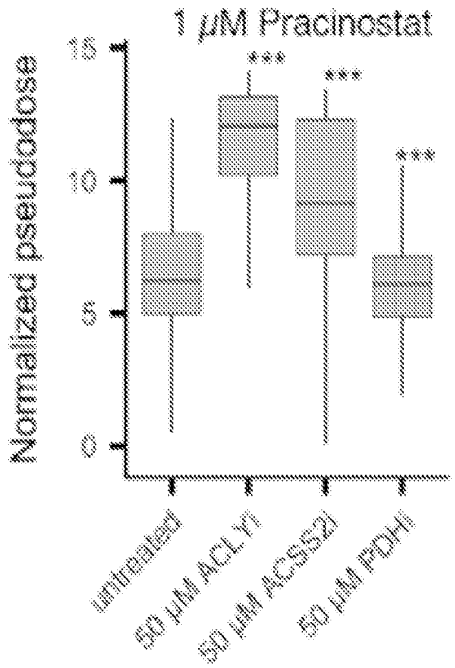


Fig. 39J

J

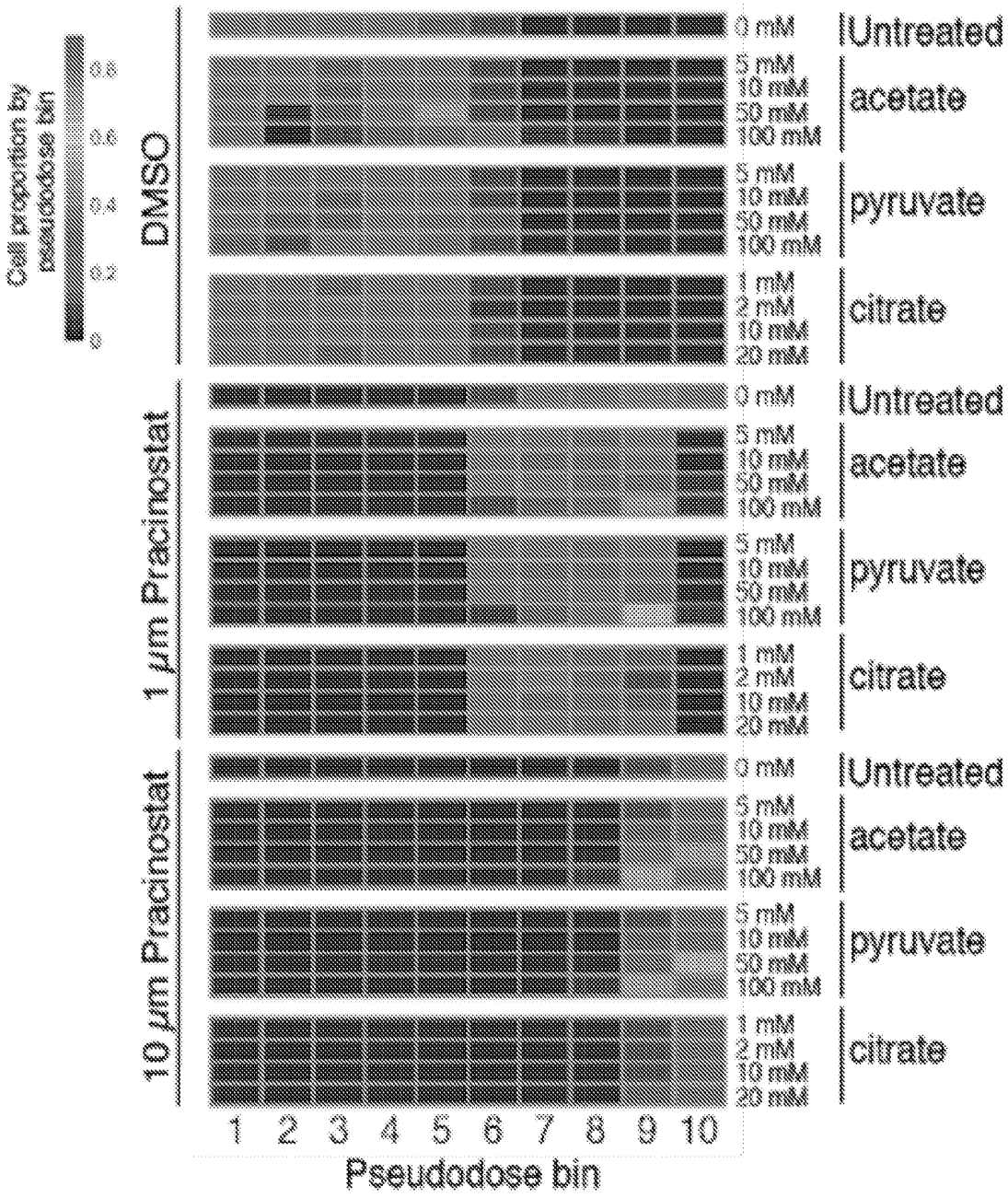

Cell proportion by pseudodose bin
0.8
0.6
0.4
0.2
0
DMSO
1 μm Pracinostat
10 μm Pracinostat
0 mM
5 mM
10 mM
50 mM
100 mM
1 mM
2 mM
10 mM
20 mM
Untreated
acetate
pyruvate
citrate
1 2 3 4 5 6 7 8 9 10
Pseudodose bin

Fig. 39K

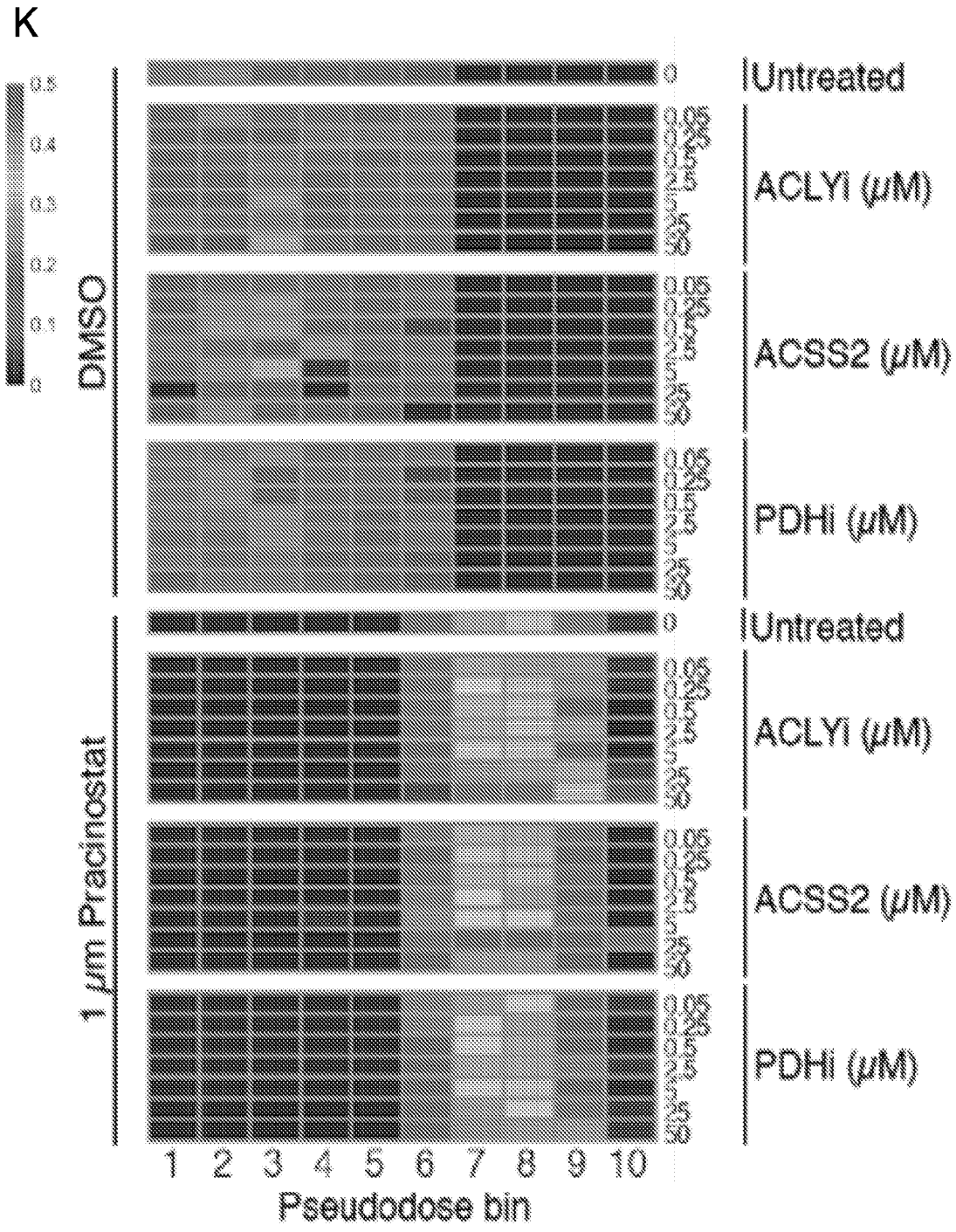

K
0.5
0.4
0.3
0.2
0.1
0
DMSO
1 μm Pracinostat
Untreated
ACLYi (μM)
ACSS2 (μM)
PDHi (μM)
0
0.05
0.25
0.5
2.5
5
25
50
1 2 3 4 5 6 7 8 9 10
Pseudodose bin

Fig. 39L

L

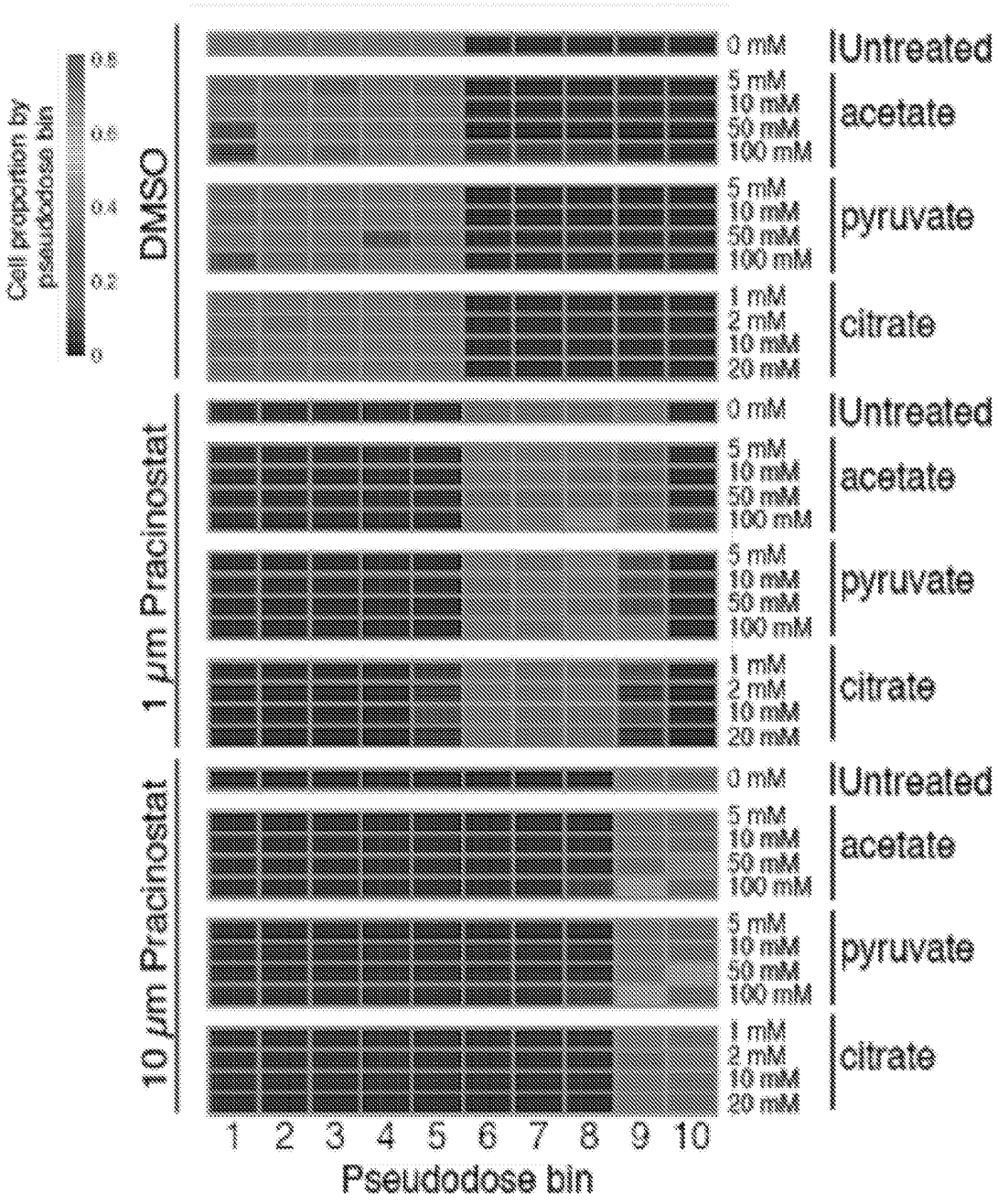

Cell proportion by pseudodose bin
0.8
0.6
0.4
0.2
0
DMSO
1 μm Pracinostat
10 μm Pracinostat
0 mM
5 mM
10 mM
50 mM
100 mM
1 mM
2 mM
10 mM
20 mM
Untreated
acetate
pyruvate
citrate
1 2 3 4 5 6 7 8 9 10
Pseudodose bin

Fig. 39M

M

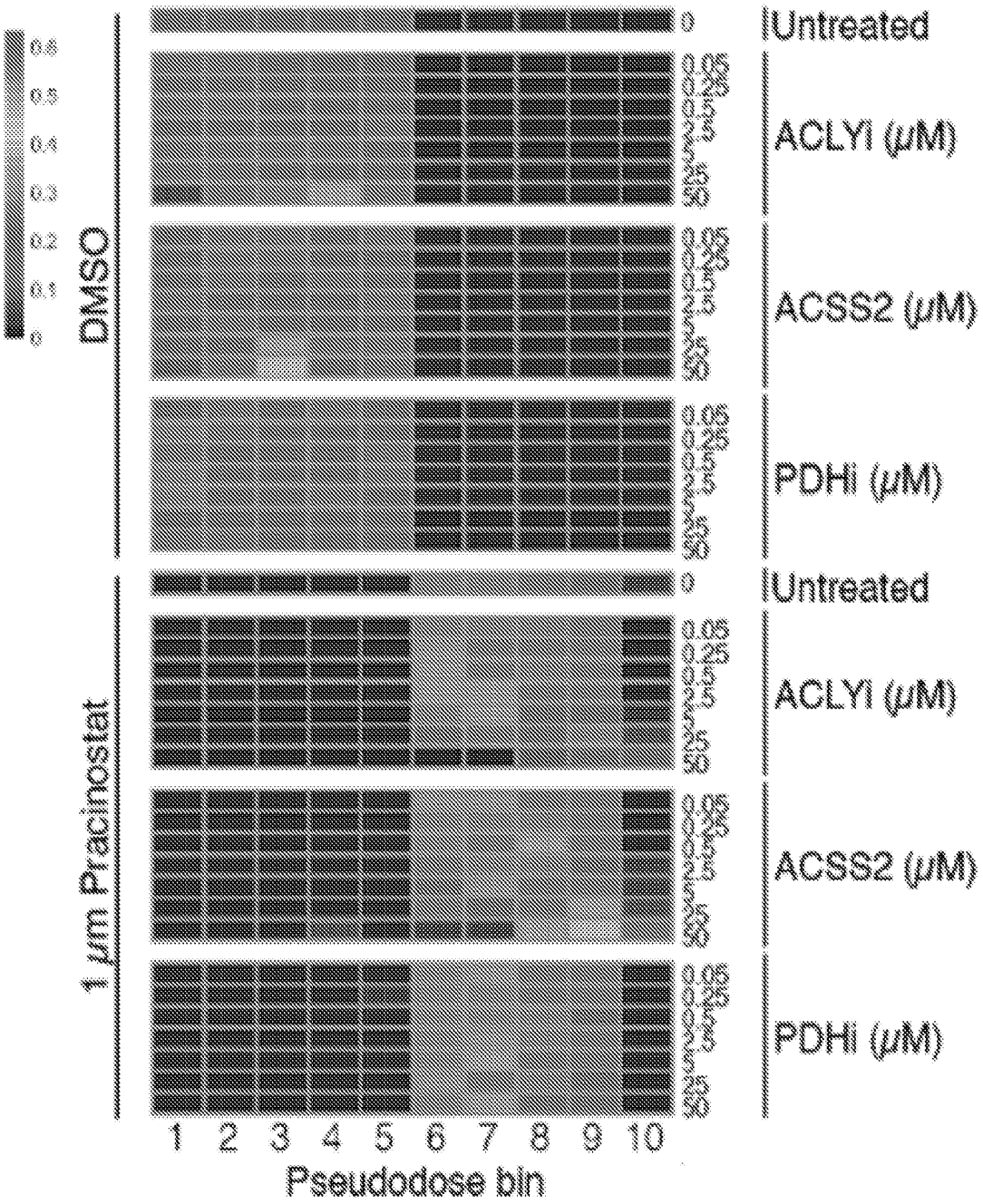

DMSO
1 μm Pracinostat
Untreated
ACLYi (μM)
ACSS2 (μM)
PDHi (μM)
0
0.05
0.25
0.5
2.5
5
25
50
1 2 3 4 5 6 7 8 9 10
Pseudodose bin

Fig. 40A

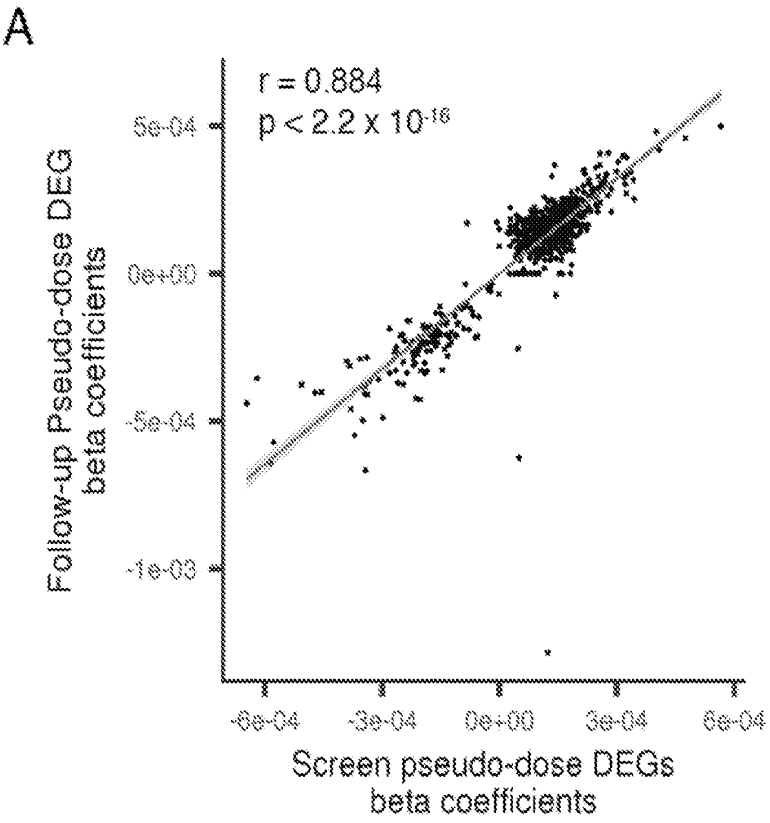


Fig. 40B

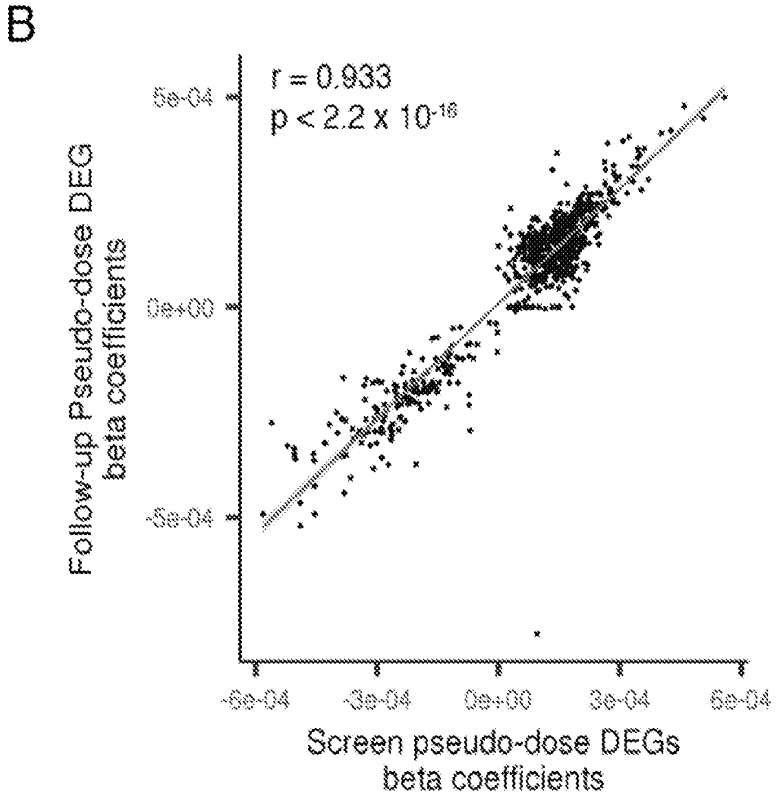


Fig. 40C

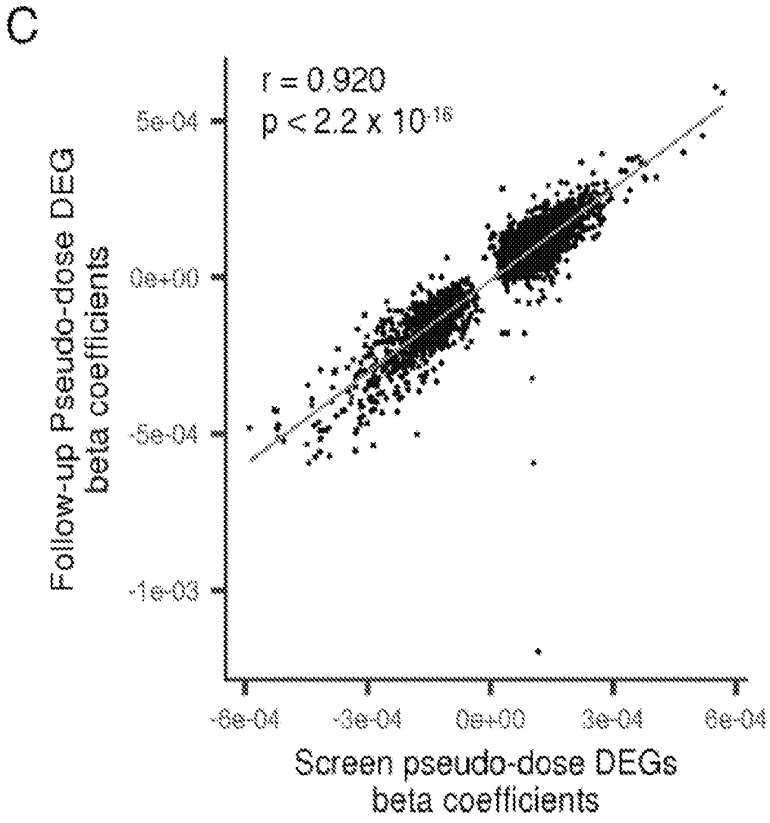
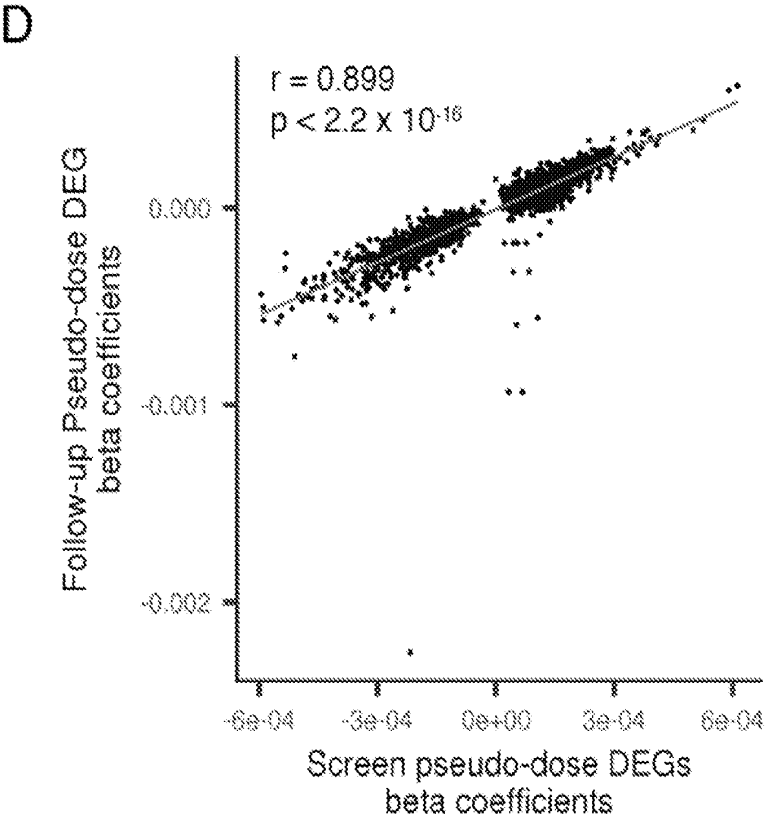


Fig. 40D



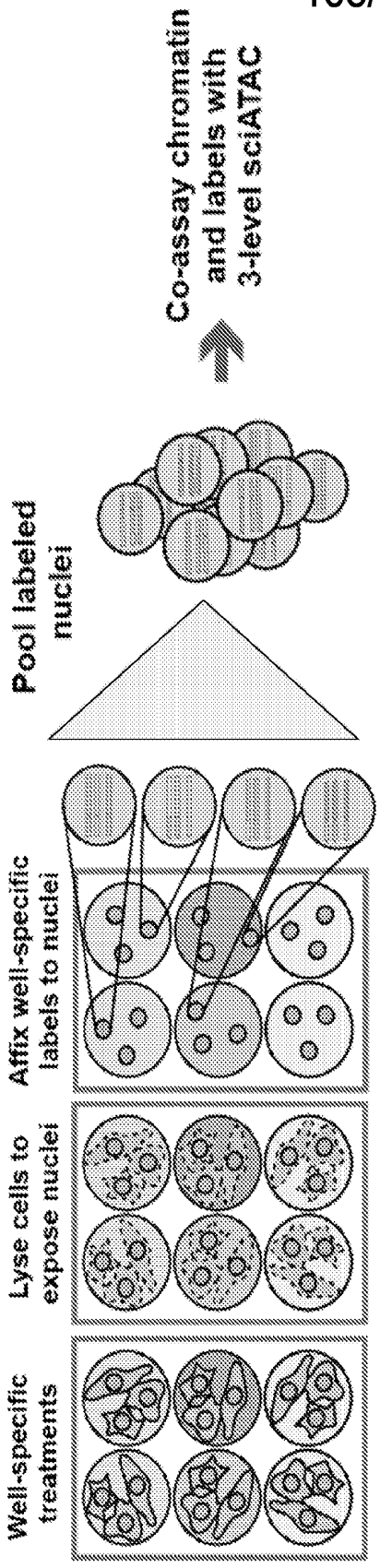

Well-specific treatments
Lyse cells to expose nuclei
Affix well-specific labels to nuclei
Pool labeled nuclei
Co-assay chromatin and labels with 3-level sciATAC

Fig. 41A
A

Fig. 41B

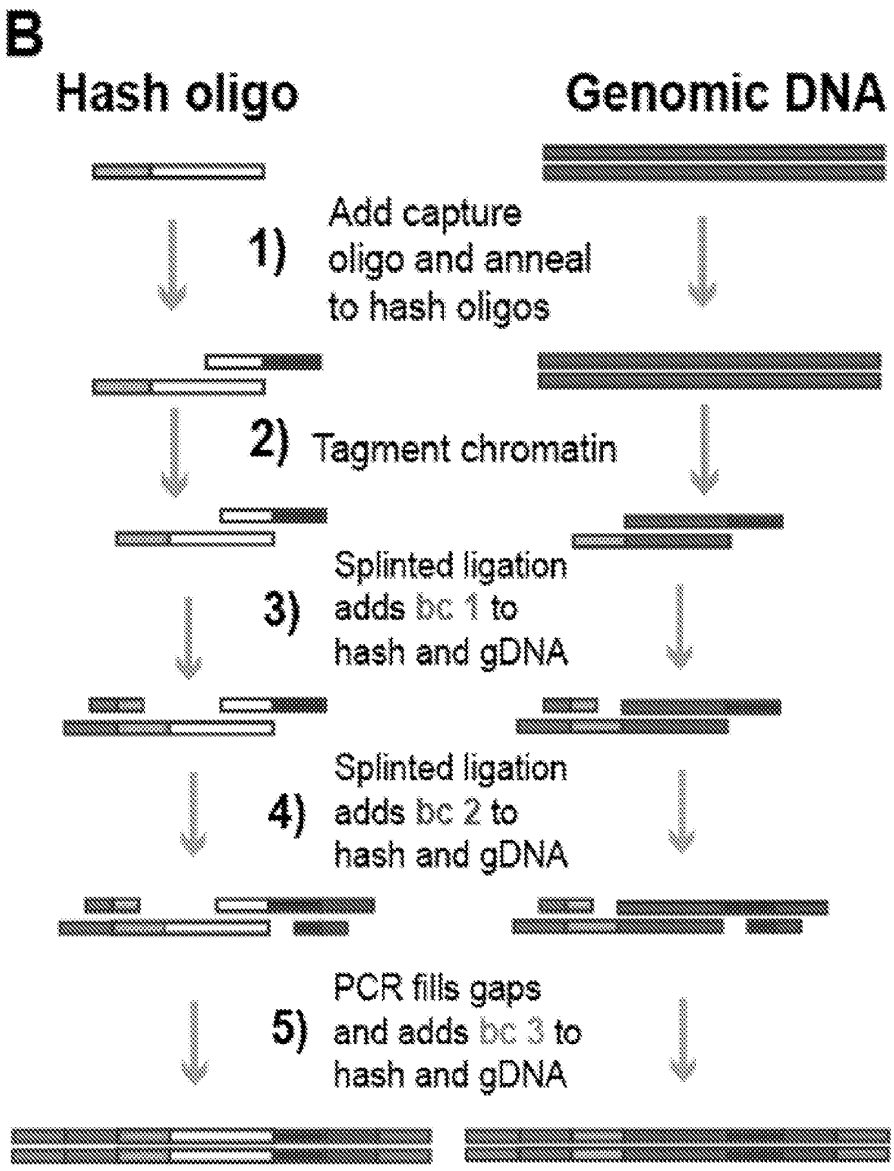

B
Hash oligo
Genomic DNA
1) Add capture oligo and anneal to hash oligos
2) Tagment chromatin
3) Splinted ligation adds bc 1 to hash and gDNA
4) Splinted ligation adds bc 2 to hash and gDNA
5) PCR fills gaps and adds bc 3 to hash and gDNA

Fig. 41C

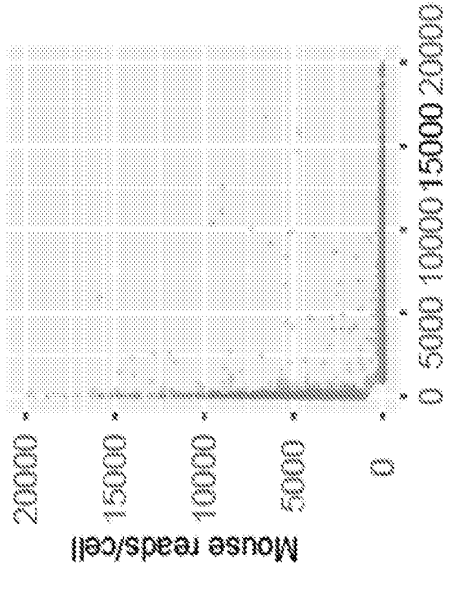


Fig. 41D

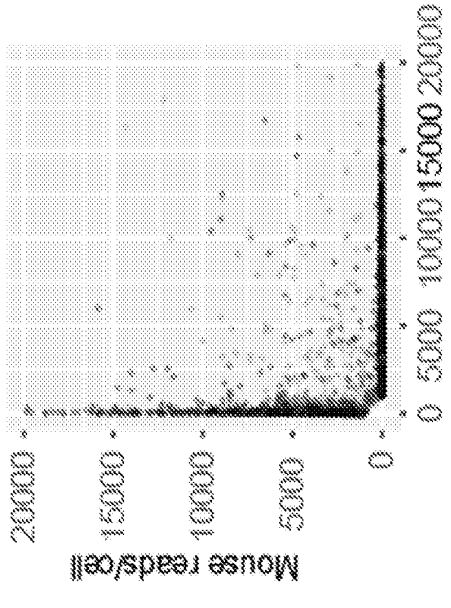


Fig. 41F

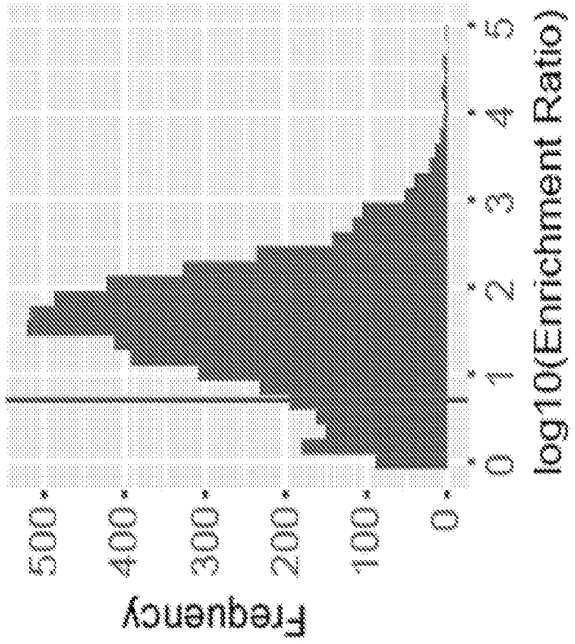


Fig. 41E

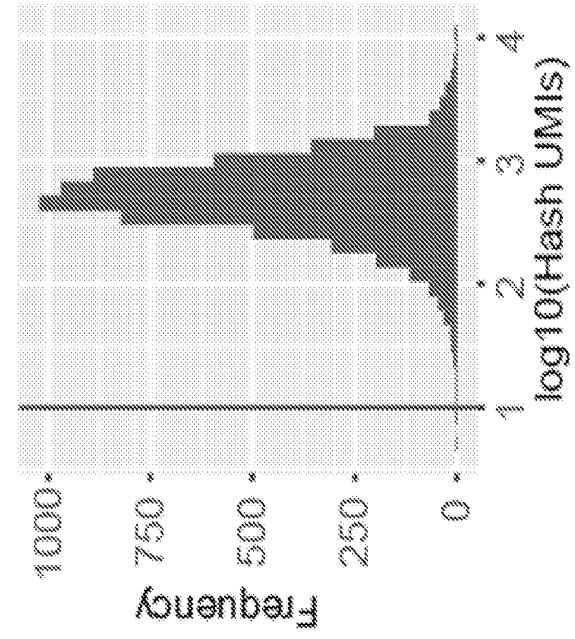


Fig. 42A

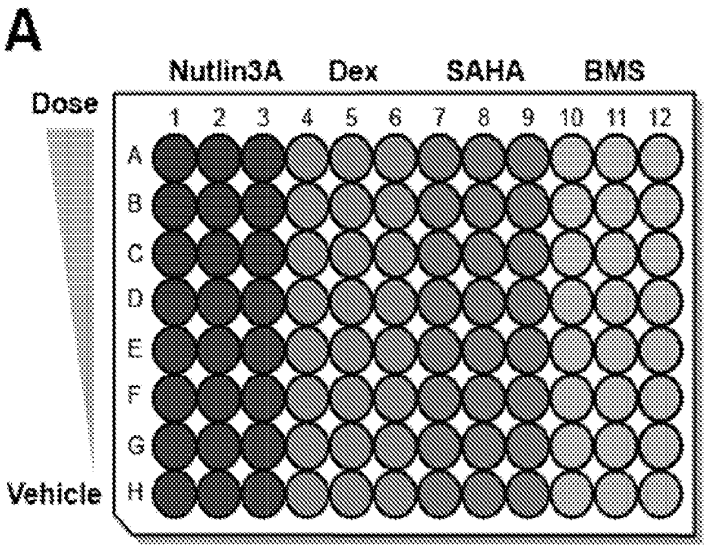
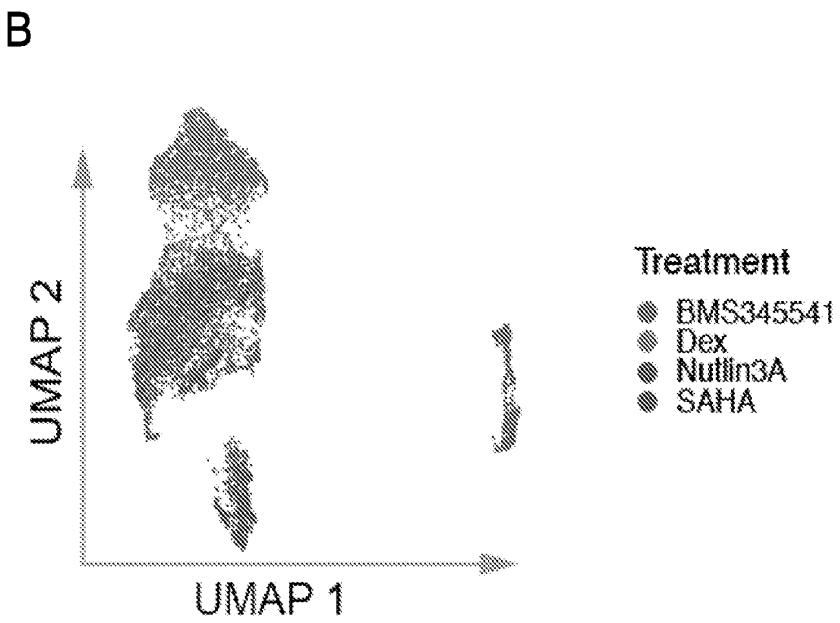


Fig. 42B



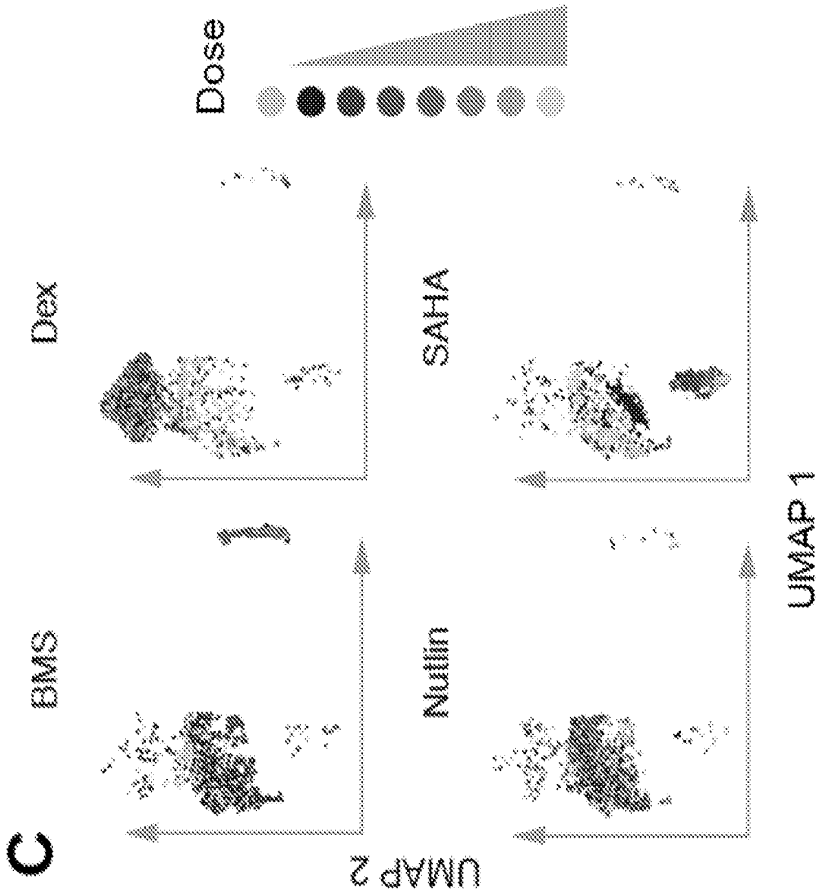


Fig. 42C

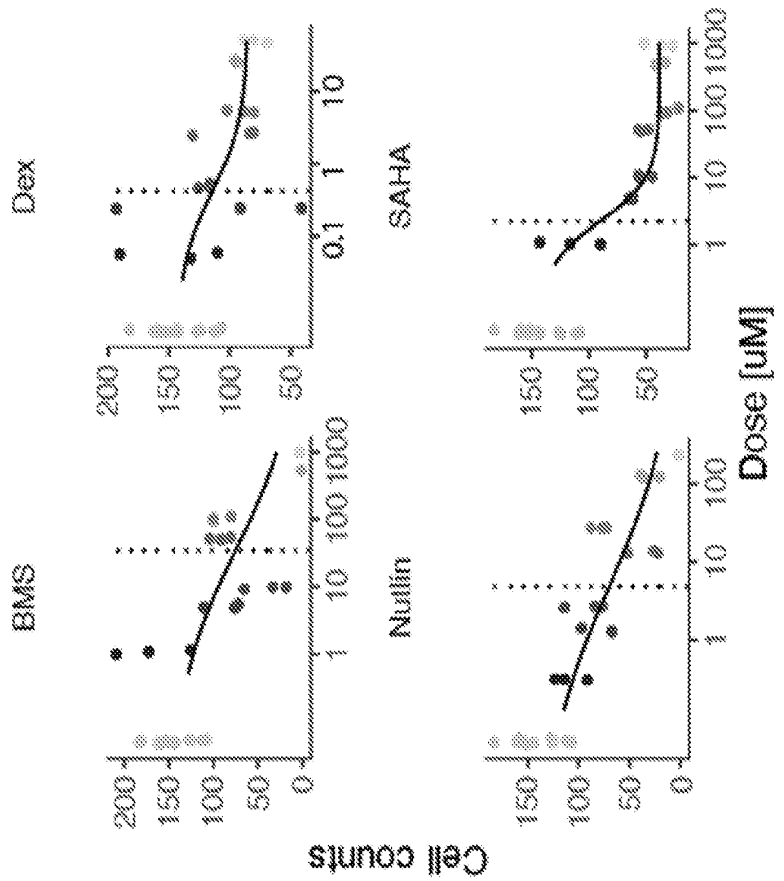
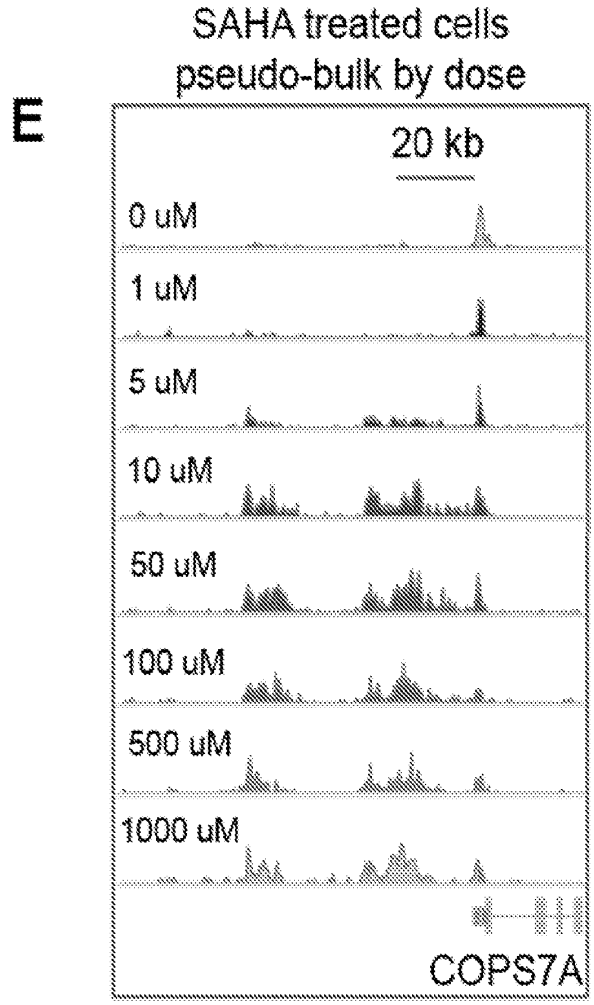

D
Dex
SAHA
BMS
Nutlin
Cell counts
Dose [uM]
200
150
100
50
0
0.1
1
10
100
1000

Fig. 42D

Fig. 42E


E
SAHA treated cells
pseudo-bulk by dose
20 kb
0 uM
1 uM
5 uM
10 uM
50 uM
100 uM
500 uM
1000 uM
COPS7A

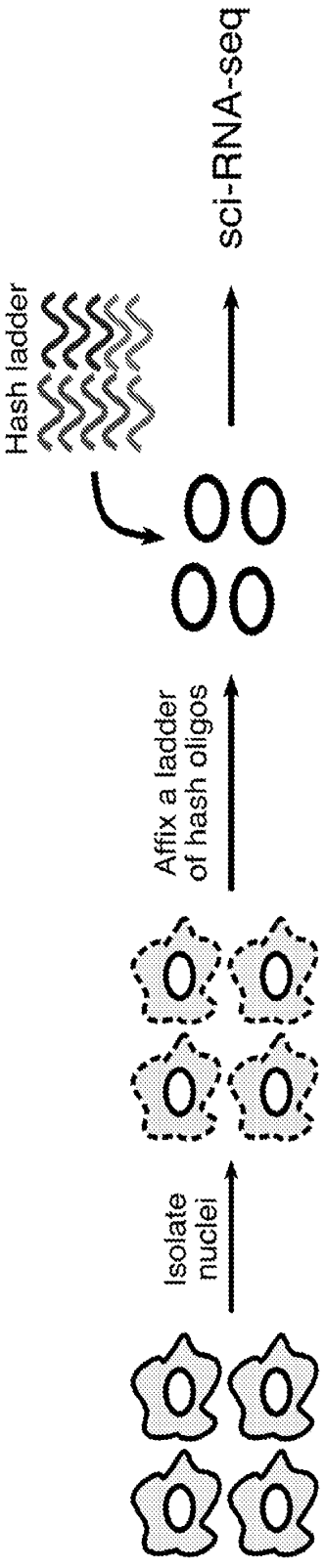


Fig. 43A

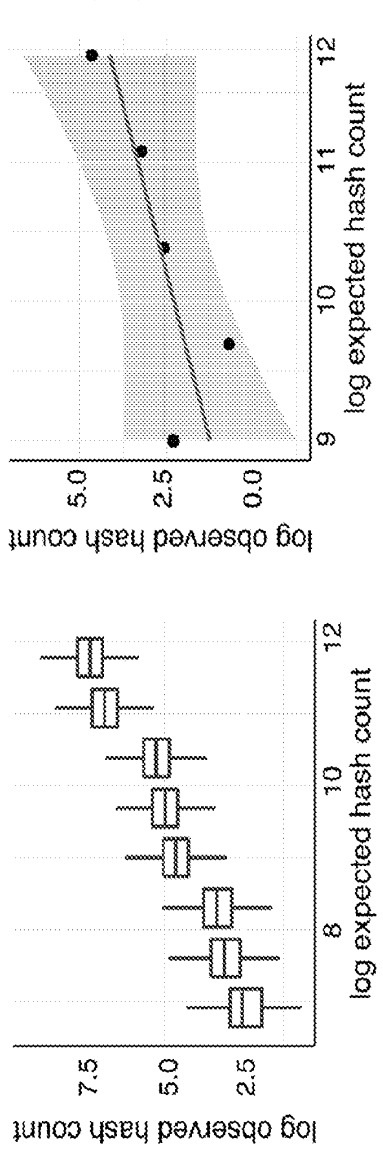


Fig. 43B

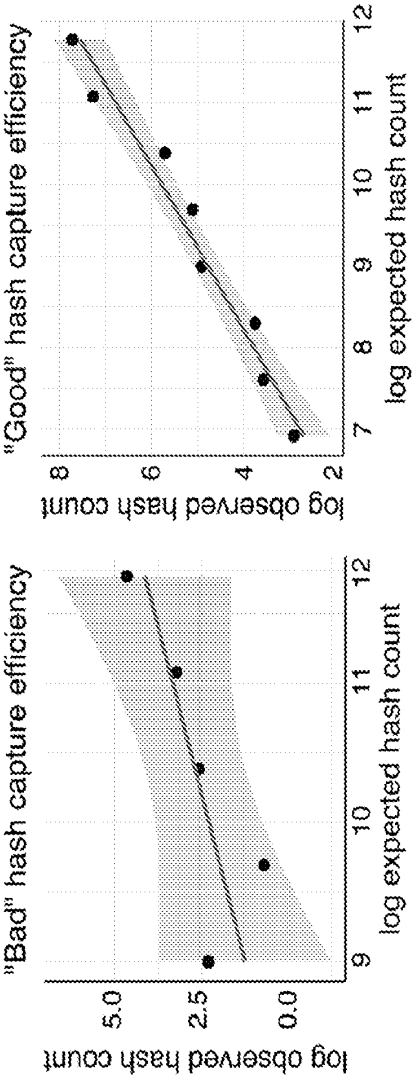


Fig. 43C

Fig. 44A

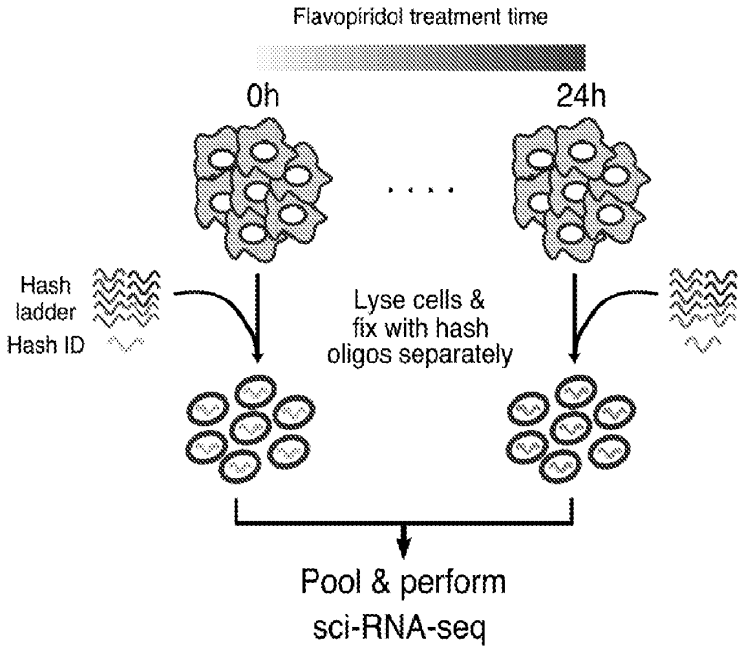


Fig. 44B

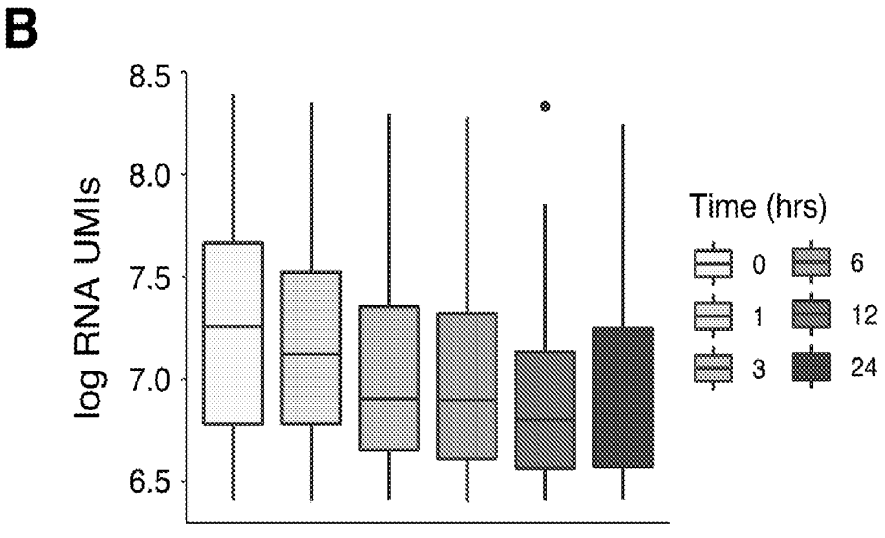


Fig. 44C

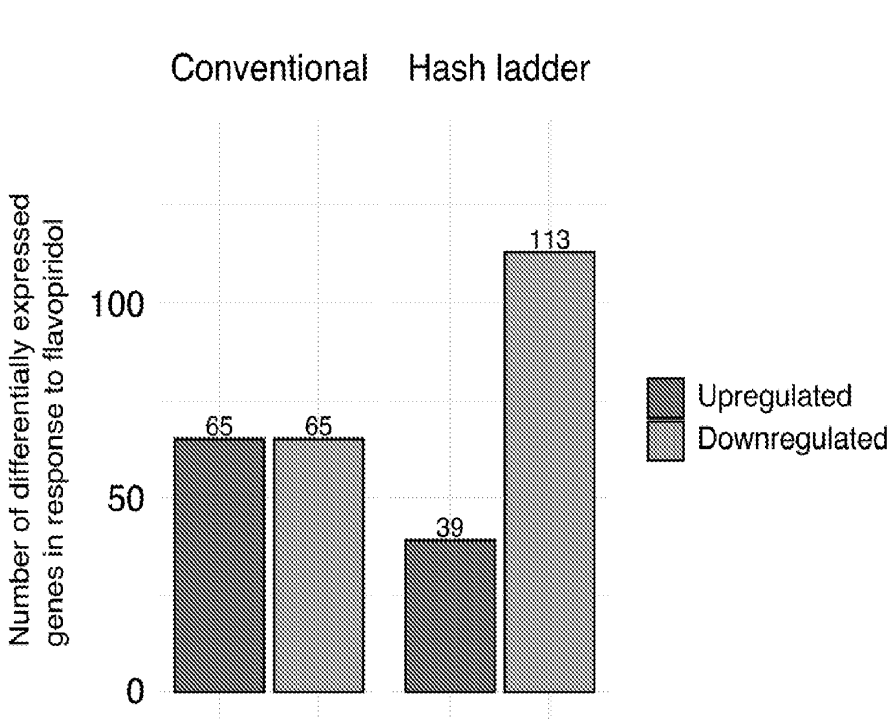


Fig. 44D

