

FIG. 1A

Cell Line	Origin	Tissue Culture Growth Conditions (+ L-Glutamine)	Source#
DU-145	Prostate	RPMI 1640, 10% FBS	ATCC
BT-549	Breast	RPMI 1640, 10% FBS	ATCC
CAL-51	Breast	DMEM, 10% FBS, 1XNEAA	DSMZ
OVCAR-3*	Ovary	RPMI 1640, 20% FBS, 0.01 mg/mL Bovine Insulin	ATCC
MDA-MB-157	Breast	RPMI 1640, 15% FBS	ATCC
MDA-MD-453	Breast	RPMI 1640, 10% FBS	ATCC
MCF-7	Breast	RPMI 1640, 10% FBS	ATCC
OVCAR-5	Ovary	RPMI 1640, 10% FBS	ACB
HCC-1954	Breast	RPMI, 10% FBS, 0.1mM NEAA	ATCC
OVCAR-8_NCI/ADR-RES	Ovary	RPMI 1640, 10% FBS	ACB
HCC-1937	Breast	RPMI 1640, 10% FBS	ATCC
ZR-75-1	Breast	RPMI 1640, 10% FBS	ATCC
HCC-1806	Breast	RPMI 1640, 10% FBS	ATCC
MAX401NLPDX	Breast	RPMI 1640, 10% FBS	CRL
OVCAR-8	Ovary	RPMI 1640, 10% FBS	ACB
HeLa	Cervix	DMEM+10% FBS+1X NEAA	ATCC

#Cell Repository Source: (ATCC) American Type Culture Collection, (ACB) Amgen Cell Bank, (DSMZ) Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH. (CRL) Charles Rivers Laboratories. *OVCAR-3 (SQ3) was selected for in vivo growth as mouse tumor xenograft at Amgen Inc.

FIG. 1B

CELL LINE	TUMOR ORIGIN	SEEDING DENSITY FOR NCA* (c/w)
DU-145	Prostate	4000
BT-549	Breast	4000
HCC-1954	Breast	4000
MDA-MB-453	Breast	6000
ZR-75-1	Breast	8000
OVCAR-5	Ovary	6000
MDA-MB-157	Breast	8000
HCC-1806	Breast	3000
OVCAR-3	Ovary	10 000
MCF-7	Breast	3000
CAL-51	Breast	2000
MX-1 (CB)	Breast	12 000 (6-day)
HCC-1937	Breast	8000, 6000 (6-day)
OVCAR-8_NCI/ADR-RES	Ovary	6000
MAX401NLPDX	Breast	5000 (6-day)
OVCAR-8	Ovary	2000

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FIG. 1C-1

KIF18A inhibitor sensitive (Yes/No)	Cell Line Name	Tissue Type	Tumor Subtype	TP53 Status	Rb pathway and CCNE1 Status	Mean Count EC ₅₀ (μM), Span >50%		
						Compound C-14, KIF18A	Palbociclib, CDK4/6	Ispinesib, Eg5
Yes	MDA-MB-157	Breast	TNBC	Mutant	CCNE1 ^{AMP}	0.0683, Yes	>10.0, No	0.0031, Yes
Yes	BT-549	Breast	TNBC	Mutant	Rb ^{DEF}	0.054, Yes	>10.0, No	0.005, Yes
Yes	OVCAR-3	Ovary	HGSOC	Mutant	CCNE1 ^{AMP}	0.0498, Yes	>10.0, No	0.0083, Yes
Yes	DU-145	Prostate	AR ^{NEG}	Mutant	Rb ^{DEF}	0.0564, Yes	>10.0, No	0.0052, Yes
Yes	HCC-1937	Breast	TNBC BRCA1 ^{MUT}	Mutant	Rb ^{DEF}	0.0493, Yes	>10.0, No	0.0026, Yes
Yes	HCC-1806	Breast	TNBC	Mutant	CCNE1 ^{AMP}	0.0665, Yes	2.712, Yes	0.0017, Yes
Yes	HCC-1954	Breast	HER2 ^{AMP} /ER ^{NEG}	Mutant	Rb ^{PROF}	0.0497, Yes	0.0713, Yes	0.0019, Yes
No	CAL-51	Breast	TNBC	Wildtype	Rb ^{PROF}	>10.0, No	0.1905, Yes	0.0017, Yes
No	MDA-MB-453	Breast	HER2 ⁷ /ER ^{NEG}	Null	Rb ^{PROF}	>10.0, No	0.2305, Yes	0.0026, Yes
No	OVCAR-5	Ovary	HGSOC	Wildtype	Rb ^{PROF}	>10.0, No	0.0467, Yes	0.0056, Yes

FIG. 1C-2

KIF18A inhibitor sensitive (Yes/No)	Cell Line Name	Tissue Type	Tumor Subtype	TP53 Status	Rb pathway and CCNE1 Status	Mean Count EC ₅₀ (μM), Span >50%		
						Compound C14, KIF18A	Palbociclib, CDK4/6	Ispinesib, Eg5
No	ZR-75-1	Breast	Luminal ER ^{POS}	Wildtype	Rb ^{PROF}	>10.0, No	0.0713, Yes	0.0029, Yes
No	MCF-7	Breast	Luminal ER ^{POS}	Wildtype	Rb ^{PROF}	0.124, No	0.0509, Yes	0.0016, Yes
	Sensitive Cell Line Mean Count EC ₅₀ (SD) Sensitive Cell Line Number					0.0563 μM (±0.008) 7	0.4818 μM (±0.9861) 7	0.0035 μM (±0.0021) 12

FIG. 2A

BT-549, TNBC
(TP53MUT, RbDEF)

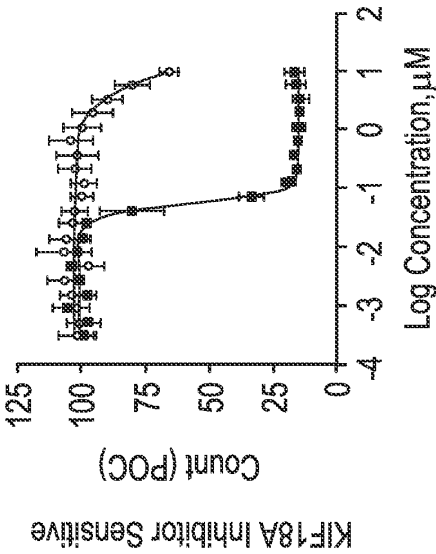


FIG. 2B

OVCAR-3, HGSO
(TP53MUT, CCNE1AMP)

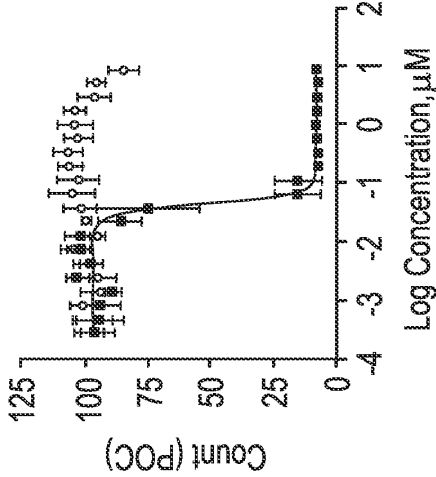


FIG. 2C

DU-145, CR-PC
(TP53MUT, RbDEF)

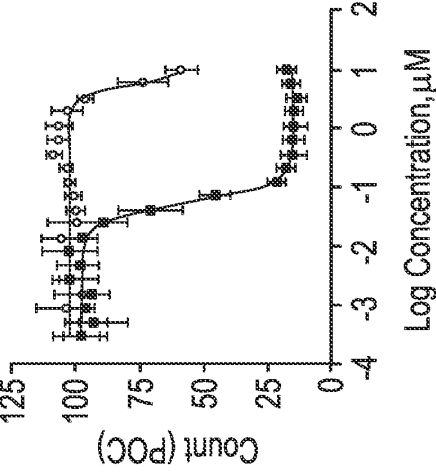


FIG. 2D

CAL-51, TNBC
(TP53 WT, Rb PROF)

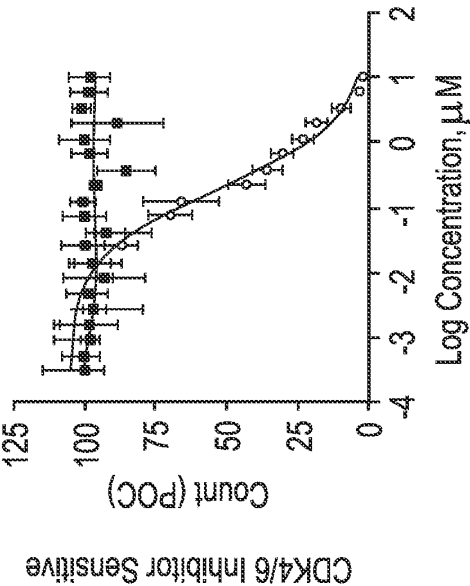


FIG. 2E

OVCAR-5, HGSOc
(TP53 WT, Rb PROF)

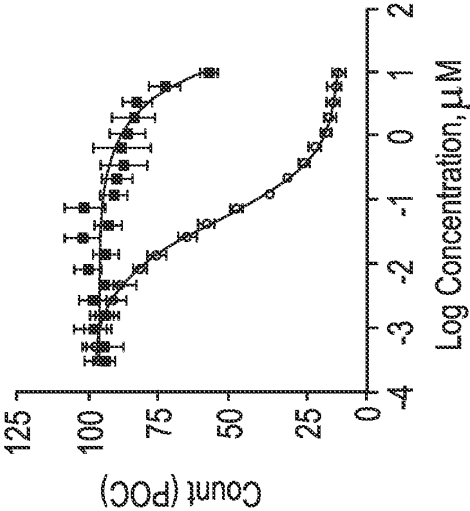
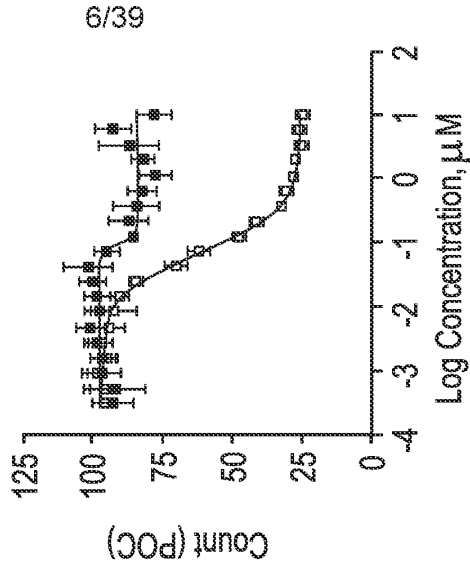


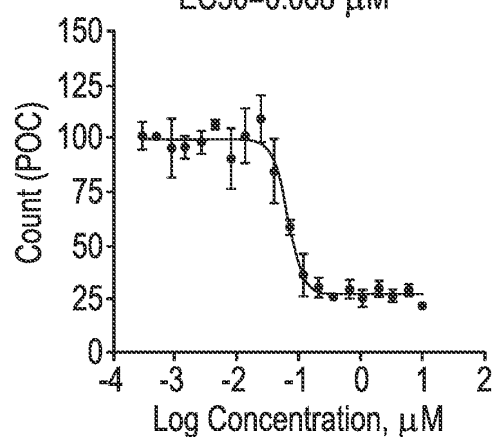
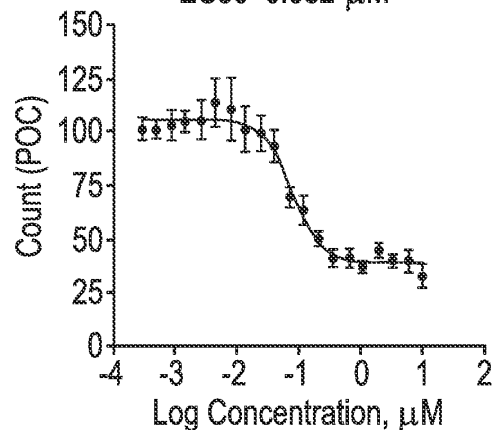
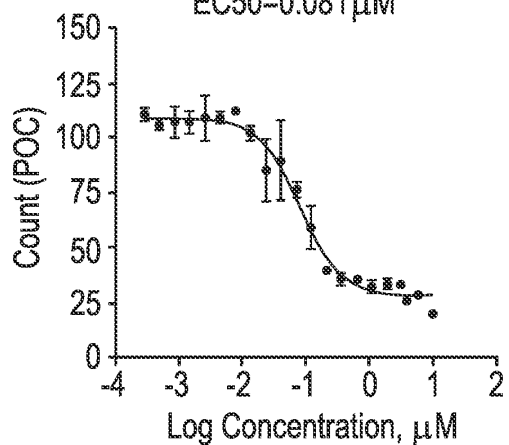
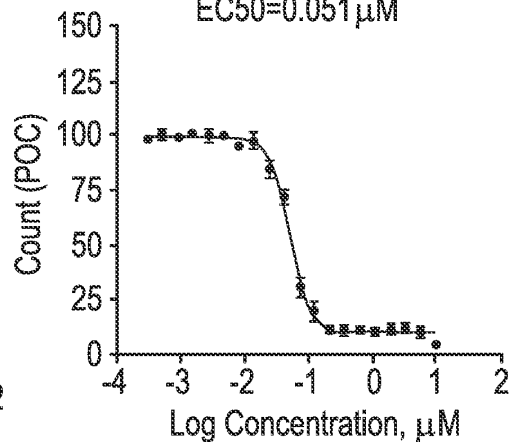
FIG. 2F

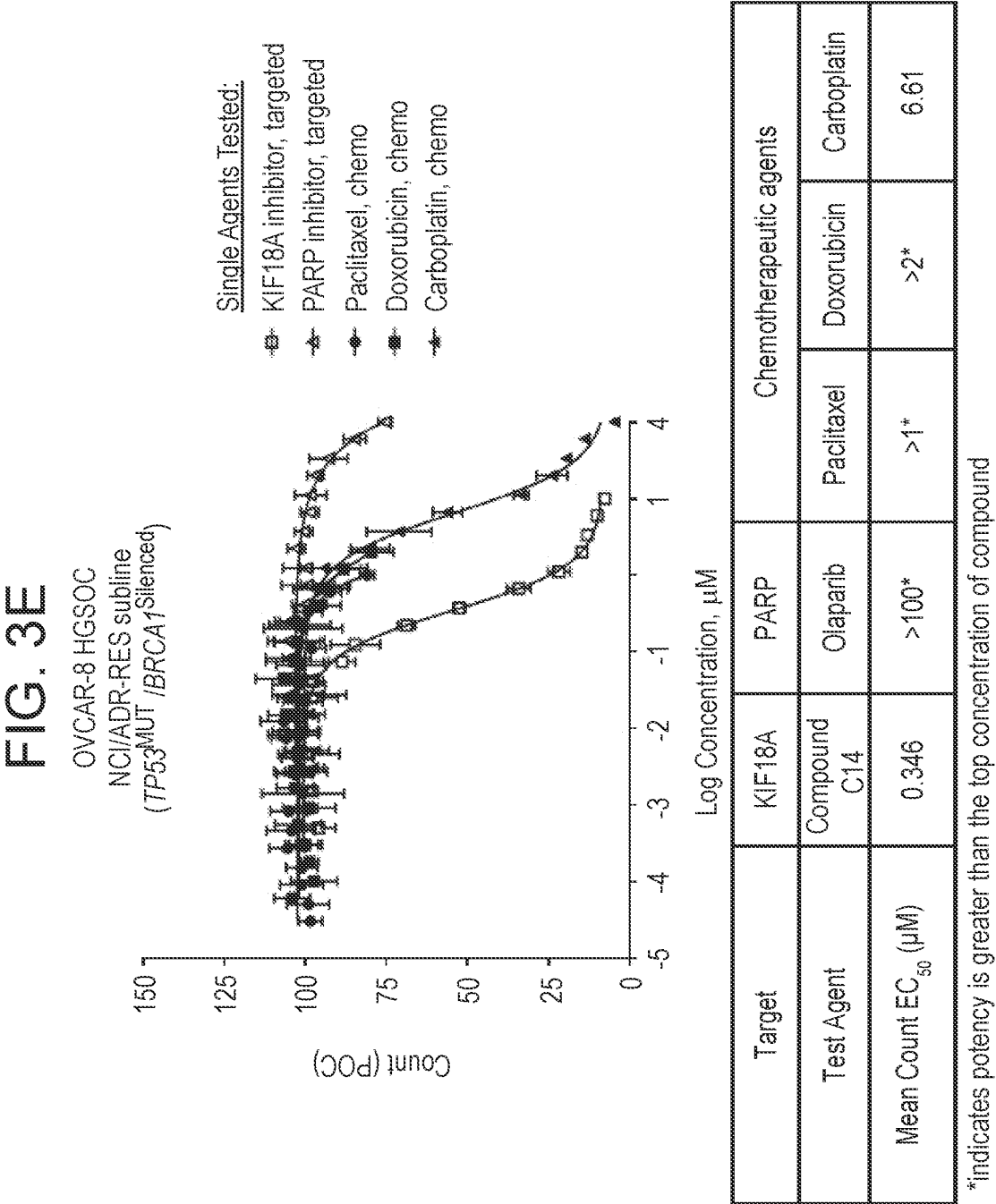
ZR-75-1, Luminal BC
(TP53 WT, Rb PROF)



■ KIF18A Inhibitor
○ CDK4/6 Inhibitor

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FIG. 3AOVCAR-8, HGSOC
(TP53 MUT/BRCA1 Silenced)4-day Count
EC50=0.068 μ M**FIG. 3B**MX-1, TNBC
(TP53 MUT/BRCA1MUT)6-day Count
EC50=0.082 μ M**FIG. 3C**MAX401NL PDX, TNBC
(TP53 MUT/BRCA1MUT)6-day Count
EC50=0.081 μ M**FIG. 3D**HCC-1937, TNBC
(TP53 MUT/BRCA1MUT)6-day Count
EC50=0.051 μ M



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FIG. 4A-1

TABLE 2

Cell line	Tumor type	Tumor Subtype	ER status	HER2	KIF18A inhibitor Sensitivity	KIF18A inhibitor, Cell EC50, uM; Screen by CP, HR, AM
AU565	BREAST	HER2	NEG	POS	Sensitive	0.016, 0.0065, ND
BT-20	BREAST	TNBC	NEG	NEG	Sensitive	ND, 0.0042, ND
BT-549	BREAST	TNBC	NEG	NEG	Sensitive	0.01424, 0.0062, 0.0183
CAL-120	BREAST	TNBC	NEG	NEG	Sensitive	ND, 0.0062, ND
HCC-1187	BREAST	TNBC	NEG	NEG	Sensitive	0.017, ND, ND
HCC-1806	BREAST	TNBC	NEG	NEG	Sensitive	0.0148, 0.0177, 0.029
HCC-1937	BREAST	TNBC	NEG	NEG	Sensitive	ND, ND, 0.0159
HCC1954	BREAST	HER2	NEG	POS	Sensitive	0.01, 0.0035, 0.01
Hs 578T	BREAST	TNBC	NEG	NEG	Sensitive	0.0134, ND, ND
JIMT-1	BREAST	HER2	NEG	POS	Sensitive	ND, 0.0036, ND
MDA-MB-157	BREAST	TNBC	NEG	NEG	Sensitive	ND, ND, 0.0225
MDA-MB-436	BREAST	TNBC	NEG	NEG	Sensitive(?)	2.0, 0.0088, ND
MDA-MB-468	BREAST	TNBC	NEG	NEG	Sensitive(?)	0.0145, 0.0095, ND
BT-474	BREAST	HER2	POS	POS	Insensitive	2.0, 0.048, ND
CAL-148	BREAST	TNBC	NEG	NEG	Insensitive	ND, 0.676, ND
CAL-51	BREAST	TNBC	NEG	NEG	Insensitive	ND, ND, 6.0
CAMA1	BREAST	LumA	POS	NEG	Insensitive	2.0, 0.236, ND
DU-4475	BREAST	TNBC	NEG	NEG	Insensitive	2.0, 0.664, ND
HCC-1143	BREAST	TNBC	NEG	NEG	Insensitive	2.0, ND, ND
HCC-1500	BREAST	LumB	POS	NEG	Insensitive	2.0, ND, ND
HCC-1569	BREAST	HER2	NEG	POS	Insensitive	2.0, ND, ND
HCC-202	BREAST	HER2	NEG	POS	Insensitive	2.0, ND, ND
HCC-2157	BREAST	TNBC	NEG	NEG	Insensitive	2.0, ND, ND
HCC-2218	BREAST	HER2	NEG	POS	Insensitive	2.0, ND, ND
HCC-38	BREAST	TNBC	NEG	NEG	Insensitive	2.0, 0.003, ND
HCC-70	BREAST	TNBC	NEG	NEG	Insensitive	2.0, 0.698, ND
HDQ-P1	BREAST	TNBC	NEG	NEG	Insensitive	ND, 2.16, ND
KPL-1	BREAST	LumA	POS	NEG	Insensitive	ND, 0.0127, ND
MCF-7	BREAST	LumA	POS	NEG	Insensitive	2.0, ND, 0.274
MDA-MB-175-VII	BREAST	LumA	POS	NEG	Insensitive	2.0, 0.0131, ND
MDA-MB-361	BREAST	HER2	POS	POS	Insensitive	2.0, 3.99, ND
MDA-MB-415	BREAST	LumA	POS	NEG	Insensitive	2.0, ND, ND
MDA-MB-453	BREAST	HER2	NEG	POS	Insensitive	2.0, 0.222, 6.0
SK-BR-3	BREAST	HER2	NEG	POS	Insensitive	ND, 0.0005, ND
SUM159PT	BREAST	TNBC	NEG	NEG	Insensitive	ND, 3.89, ND
T47D	BREAST	LumA	POS	NEG	Insensitive	ND, 0.0082, ND
UACC-812	BREAST	LumB	POS	POS	Insensitive	2.0, ND, ND
UACC-893	BREAST	HER2	NEG	POS	Insensitive	2.0, ND, ND
ZR-75-1	BREAST	LumA	POS	NEG	Insensitive	2.0, 0.003, 0.052
ZR-75-30	BREAST	HER2	POS	POS	Insensitive	2.0, 1.94, ND

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FIG. 4A-2
TABLE 2

KIF18A inhibitor, Cell Span or Max Response; Screen by CP, HR, AM	Consensus TP53 Status	TP53 Variant Classification	p53 Protein Change
66.4, 82.6, ND	MUT	MM	p.R175H
ND, 59.7, ND	MUT	MM	p.K132Q
70.1, 87.8, 81.9	MUT	MM	p.R249S
ND, 63.9, ND	MUT	SS	NA
76.5, ND, ND	MUT	IFD	p.G108del
78.6, 77.8, 87.8	MUT	FSI	p.T256fs
ND, ND, 69.9	MUT	NM	p.R306*
52.4, 80.6, 65	MUT	MM	p.Y163C
84.6, ND, ND	MUT	MM	p.V157F
ND, 87.9, ND	MUT	MM	p.R248W
ND, ND, 62.6	MUT	FSD	p.APSWPLSSS88fs
19.0, 61.8, ND	MUT	FSI	p.E204fs
42.0, 52.6, ND	MUT	MM	p.R273H
7.6, 33.9, ND	MUT	MM	p.E285K
ND, 33.5, ND	MUT	MM	p.E224K
ND, ND, 28.2	WT		
1.57, 29.8, ND	MUT	MM	p.R280T
4.72, 23.5, ND	WT		
20.6, ND, ND	MUT	MM	p.R248Q
12.5, ND, ND	WT		
4.2, ND, ND	MUT	NM	p.E294*
2.9, ND, ND	MUT	FSD	p.T284fs
5.1, ND, ND	MUT	MM	p.R248W
2.9, ND, ND	?	MM	p.R283C
20.4, 32.3, ND	MUT	MM	p.R273L
19.2, 6.8, ND	MUT	MM	p.R248Q
ND, 26.5, ND	MUT	NM	p.R213*
ND, 33.5, ND	WT		
3.5, ND, 31.6	WT		
3.73, 34.6, ND	WT		
9.0, 15.1, ND	MUT	NM	p.E56*
25.5, ND, ND	MUT	MM	p.Y236C
11.7, 32.1, 27.3	NULL	NULL	
ND, 28.7, ND	MUT	MM	p.R175H
ND, 2.82, ND	MUT	IFI	p.157_158insL
ND, 42.0, ND	MUT	MM	p.L194F
6.2, ND, ND	WT		
3.9, ND, ND	MUT	NM	p.R342*
-1.5, 23.9, 24.1	WT		
12.4, 16.5, ND	WT		

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FIG. 4B-1
TABLE 3

Cell line	Tumor type	Tumor Subtype	KIF18A inhibitor Sensitivity	KIF18A inhibitor, Cell EC50, uM; Screen by CP, HR, AM
COV362	OVARY	Likely HGSOC	Sensitive(?)	0.0168, 0.007, ND
ES-2	OVARY	Possible HGSOC	Sensitive	0.0249, 0.0191, ND
JHOM-1	OVARY	Possible HGSOC	Sensitive	ND, 0.0167, ND
JHOM-2B	OVARY	Possible HGSOC	Sensitive	ND, 0.0116, ND
KURAMOCHI	OVARY	Likely HGSOC	Sensitive	0.0123, 0.0084, ND
MCAS	OVARY	Unlikely HGSOC	Sensitive(?)	0.0259, 0.0934, ND
NCI-ADR-RES	OVARY	Possible HGSOC	Sensitive	ND, 0.0365, ND
OAW-28	OVARY	Likely HGSOC	Sensitive	ND, 0.0027, ND
OC-316	OVARY	Hypermutated	Sensitive	ND, 0.006, ND
ONCO-DG-1	OVARY	Possible HGSOC	Sensitive	ND, 0.0055, ND
OVCAR-3	OVARY	Possible HGSOC	Sensitive	0.0086, 0.0039, 0.0201
OVCAR-8	OVARY	Possible HGSOC	Sensitive	ND, 0.0126, 0.0223
OVSCHO	OVARY	Likely HGSOC	Sensitive	ND, 0.0108, ND
SNU-119	OVARY	Likely HGSOC	Sensitive	ND, 0.0071, ND
A2780	OVARY	Unlikely HGSOC	Insensitive	0.054, ND, ND
Caov-3	OVARY	Likely HGSOC	Insensitive	2.0, 3.99, ND
Caov-4	OVARY	Likely HGSOC	Insensitive	ND, 3.99, ND
COV434	OVARY	Unlikely HGSOC	Insensitive	2.0, 0.135, ND
COV504	OVARY	Possible HGSOC	Insensitive	ND, 0.0033, ND
COV644	OVARY	Unlikely HGSOC	Insensitive	2.0, 0.568, ND
EFO-21	OVARY	Unlikely HGSOC	Insensitive	ND, 0.042, ND
EFO-27	OVARY	Hypermutated	Insensitive	ND, 0.0224, ND
IGROV-1	OVARY	Hypermutated	Insensitive	ND, 0.0894, ND
JHOC-5	OVARY	Unlikely HGSOC	Insensitive	ND, 1.68, ND
OAW-42	OVARY	Unlikely HGSOC	Insensitive	2.0, 1.66, ND
OV56	OVARY	Unlikely HGSOC	Insensitive	2.0, 0.371, ND
OV90	OVARY	Possible HGSOC	Insensitive	2.0, 0.0178, ND
OVCAR-4	OVARY	Likely HGSOC	Insensitive	ND, 0.0622, ND
OVCAR-5	OVARY	?	Insensitive	ND, 0.0110, 0.280
OVISE	OVARY	Unlikely HGSOC	Insensitive	ND, 0.029, ND
OVK18	OVARY	Hypermutated	Insensitive	ND, 0.4369, ND
OVMANA	OVARY	Unlikely HGSOC	Insensitive	1.0, ND, ND
OVTOKO	OVARY	Unlikely HGSOC	Insensitive	2.0, 0.001, ND
PA-1	OVARY	?	Insensitive	ND, 0.067, ND
RKN	OVARY	?	Insensitive	2.0, ND, ND
RMUG-S	OVARY	Possible HGSOC	Insensitive	2.0, 0.0003, ND
SK-OV-3	OVARY	Unlikely HGSOC	Insensitive	ND, 0.0165, ND
SNU-840	OVARY	Unlikely HGSOC	Insensitive	2.0, 0.054, ND
TOV-21G	OVARY	Hypermutated	Insensitive	ND, 0.074, ND
TYK-nu	OVARY	Likely HGSOC	Insensitive	ND, 0.107, ND

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FIG. 4B-2

TABLE 3

KIF18A inhibitor, Cell Span or Max Response; Screen by CP, HR, AM	Consensus TP53 Status	TP53 Variant Classification	p53 Protein Change
59.3, 46.8	MUT	MM	p.Y220C
58.5, 71.0, ND	MUT	MM	p.S241F
ND, 83.6, ND	MUT	NM	p.R213*
ND, 64.3, ND	MUT	MM	p.C275F
49.5, 88.4, ND	MUT	MM	p.D281Y
59.7, 39.1, ND	MUT	MM	p.L114F
ND, 95.5, ND	MUT	SS	
ND, 77.8, ND	MUT	FSD	p.P153fs
ND, 95.2, ND	MUT	MM	p.R273H
ND, 97.4, ND	MUT	MM	p.R248Q
78.6, 97.6, 88.2	MUT	MM	p.R248Q
ND, 89.1, 81.3	MUT	SS	
ND, 68.6, ND	MUT	NM	p.R342*
ND, 81.9, ND	MUT	MM	p.P151A
33.0, ND, ND	WT		
12.3, 15.2, ND	MUT	NM	p.Q136*
ND, 1.34, ND	MUT	MM	p.V147D
-0.67, 11.9, ND	WT		
ND, 42.8, ND	MUT	FSD	p.L323fs
16.4, 30.8	LOE	SS	NA
ND, 17.4, ND	MUT	MM	p.C124R
ND, 9.56, ND	MUT	MM	p.R273C
ND, 25.4, ND	MUT	SS	p.Y126C
ND, 14.4, ND	WT		
11.3, 14.2, ND	WT		
-0.209, 34.8, ND	MUT	FSD	p.KT101fs
1.61, 43.8, ND	MUT	MM	p.S215R
ND, 15.8, ND	MUT	MM	p.L130V
ND, 26.7, 16.7	MUT/WT	MM	p.R273C
ND, 19.4, ND	WT		
ND, 6.73, ND	MUT	FSD	p.P153fs
14.5, ND, ND	WT		
11.4, 14.5, ND	WT		
ND, 23.2, ND	MUT/WT	S	p.P316P
25.3, ND, ND	MUT	MM	p.R175H
7.3, 21.6, ND	MUT	MM	p.A347V
ND, 13.7, ND	MUT	FSD	p.P89fs
3.25, 29.4, ND	WT		
ND, 42.0, ND	WT		
ND, 22.6, ND	MUT	MM	p.R175H

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FIG. 4C
KIF18A Inhibitor Sensitive Group

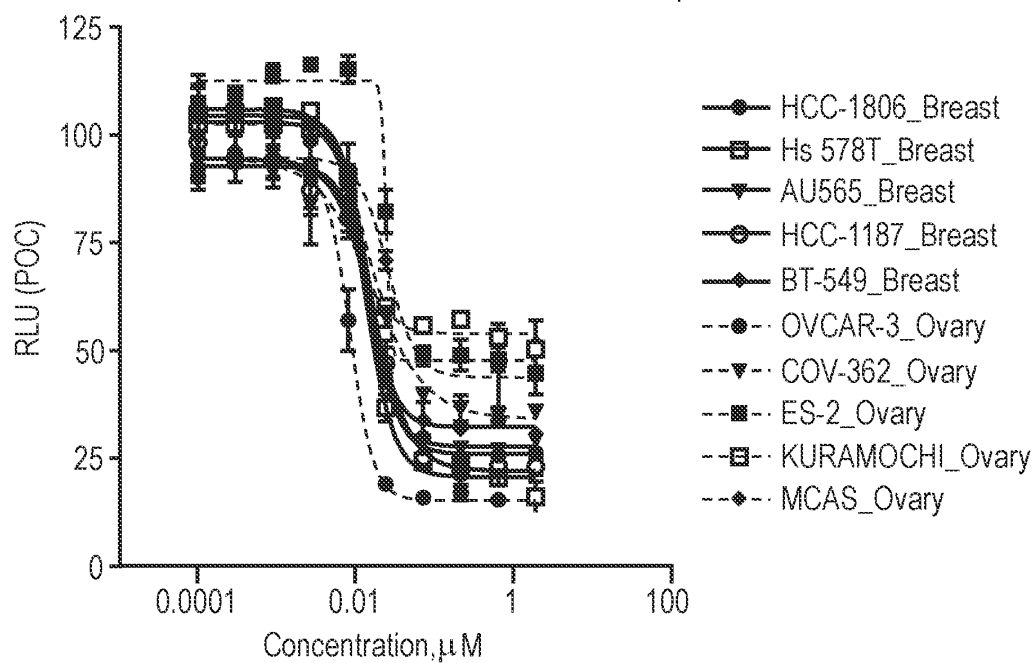
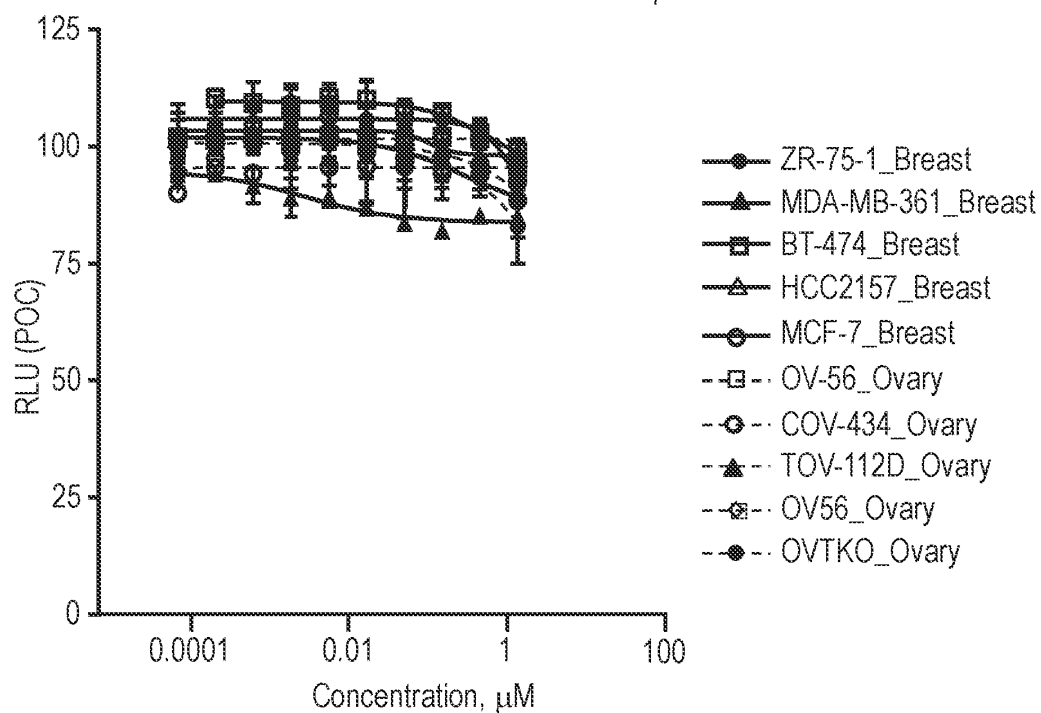


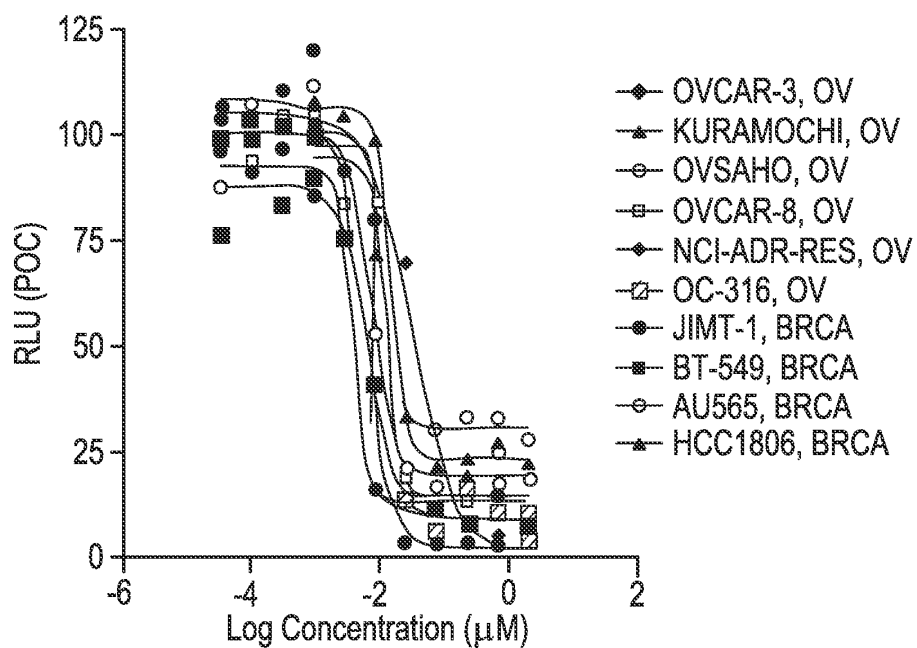
FIG. 4D
KIF18A Inhibitor Insensitive Group



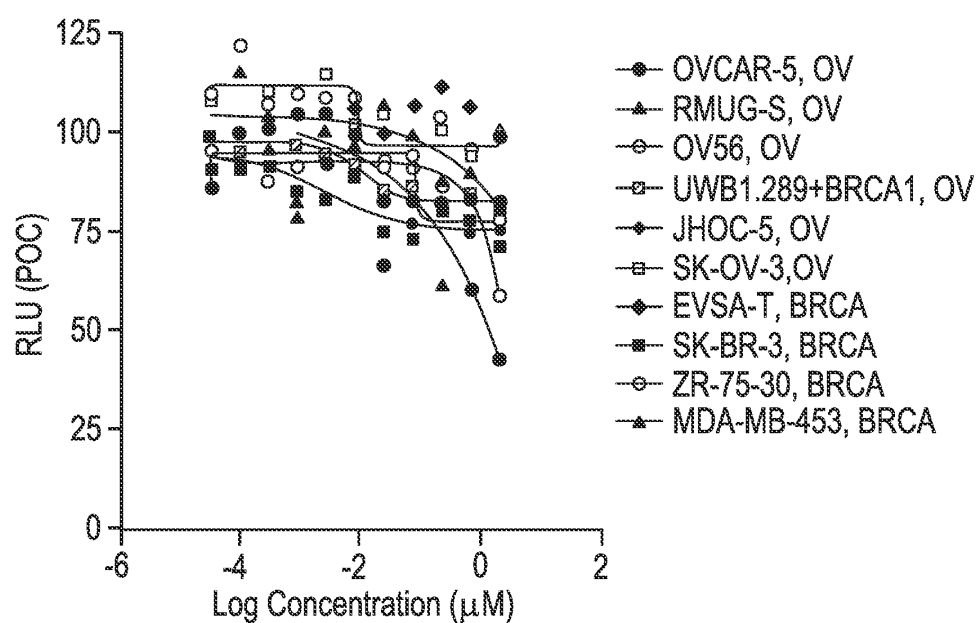
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FIG. 4E

KIF18A Inhibitor Sensitive Group

**FIG. 4F**

KIF18A Inhibitor Insensitive Group



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FIG. 5B

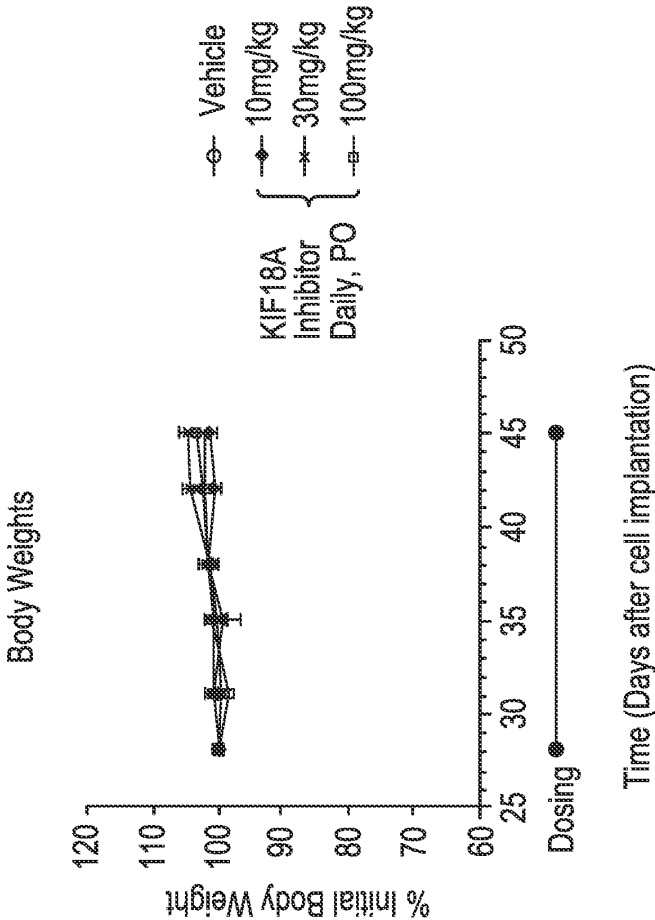


FIG. 5A

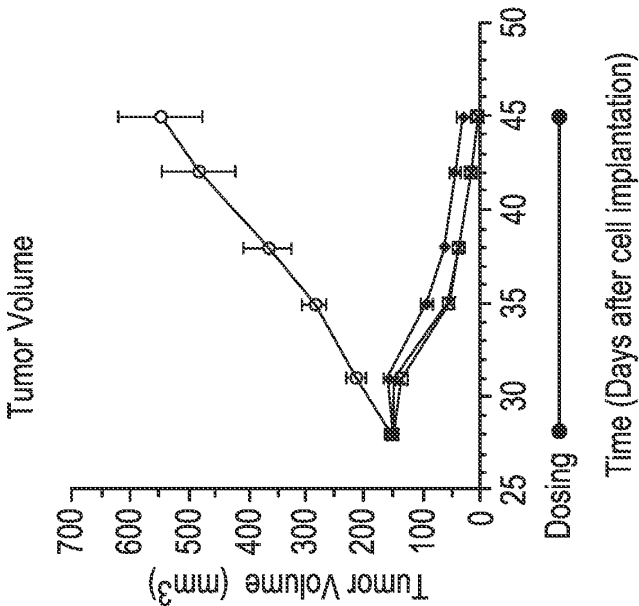


FIG. 6A

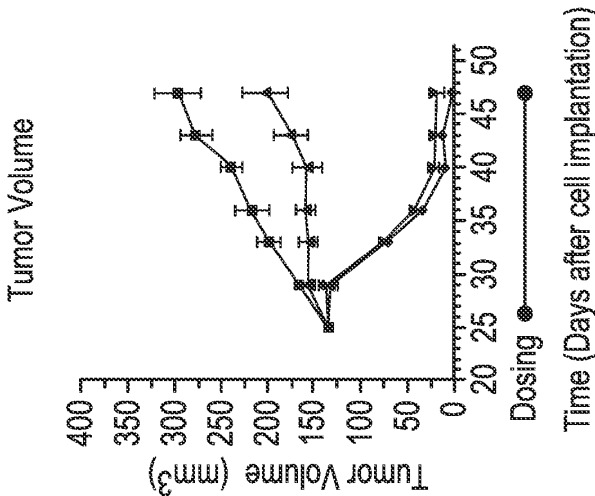
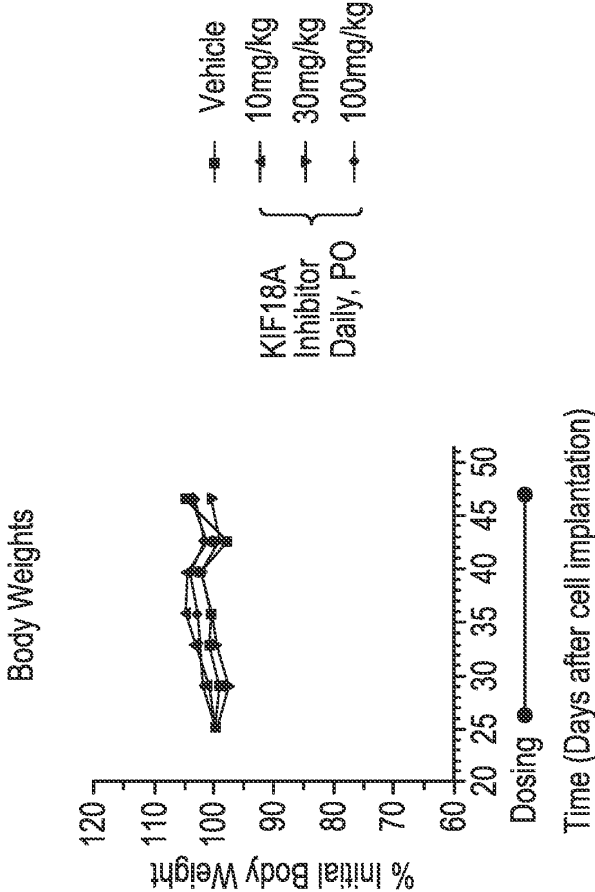


FIG. 6B



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FIG. 7A

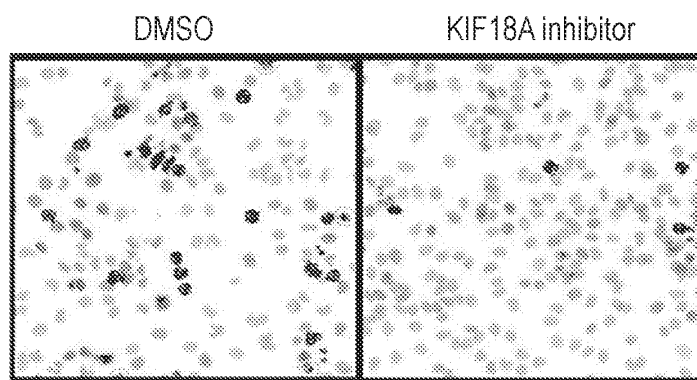


FIG. 7B

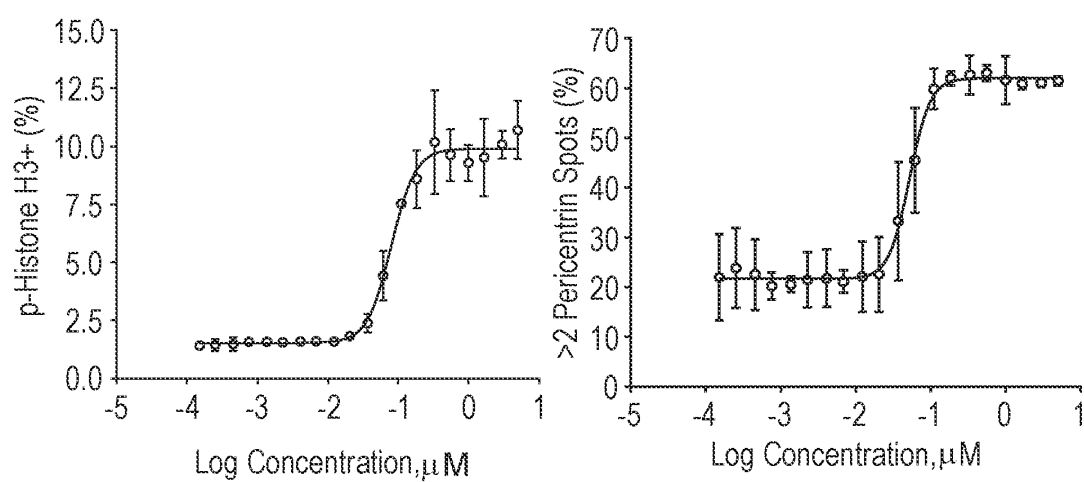


FIG. 7C

Cellular Endpoint	Mean EC50 (μM)	SD
>2 Pericentrin Spot Count	0.0522	± 0.0138
p-Histone H3	0.0794	± 0.0196

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FIG. 8A

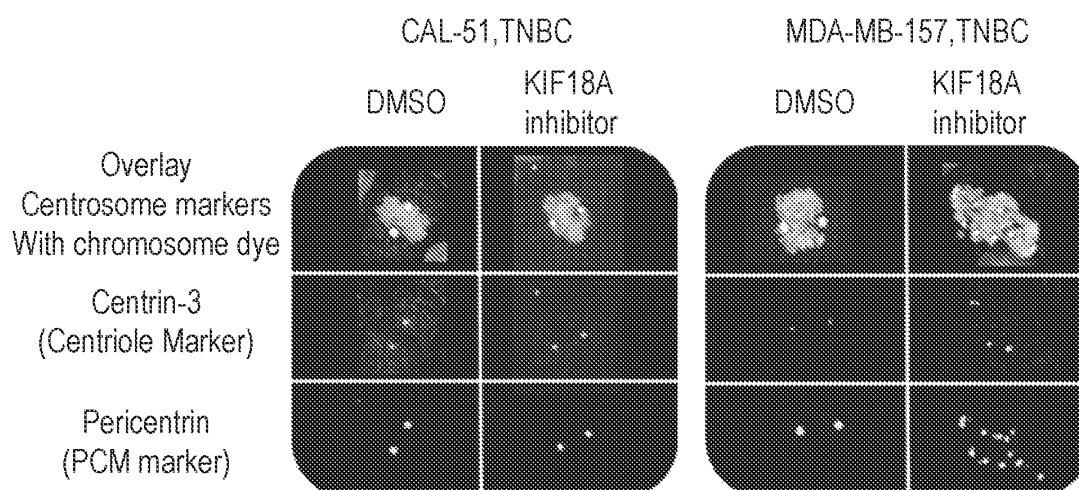


FIG. 8B

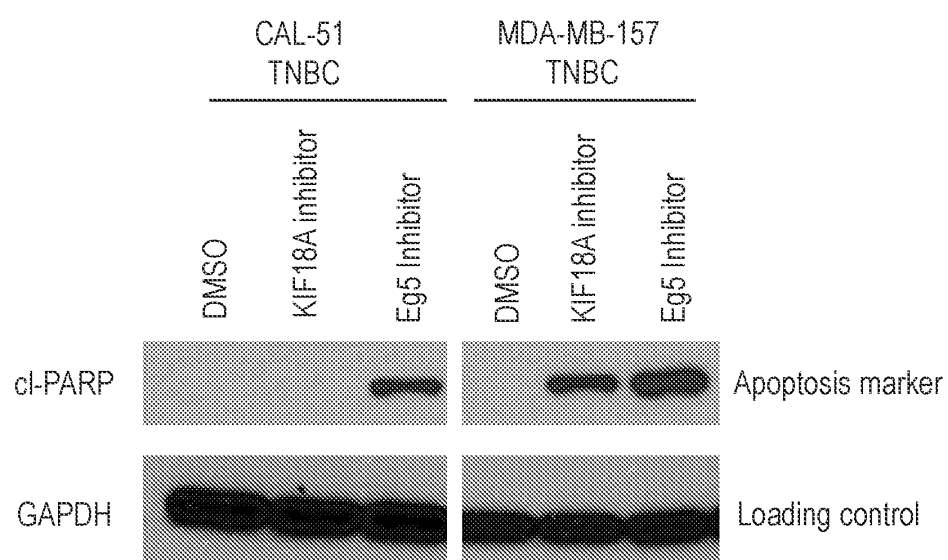
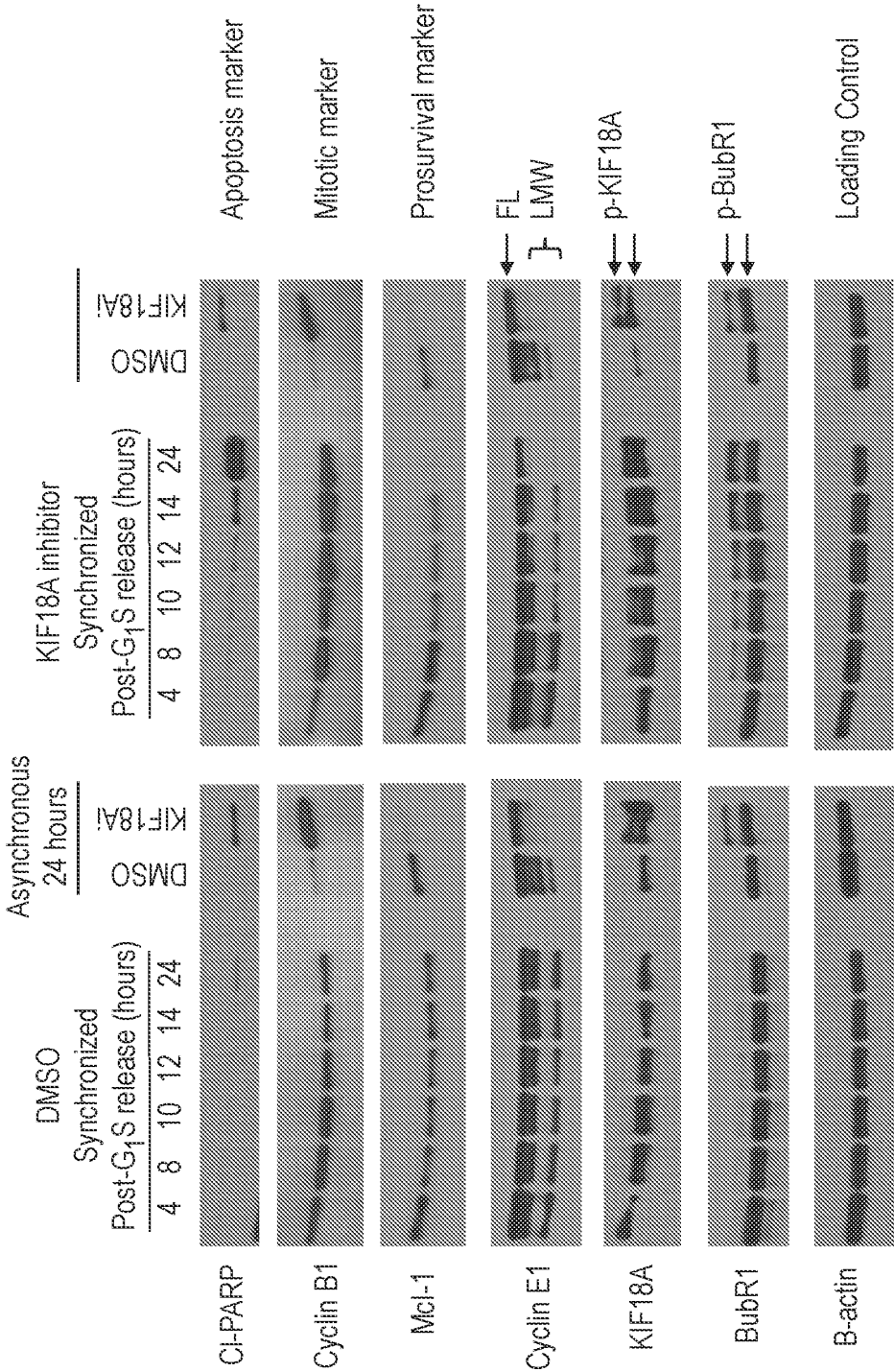


FIG. 9



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FIG. 10A

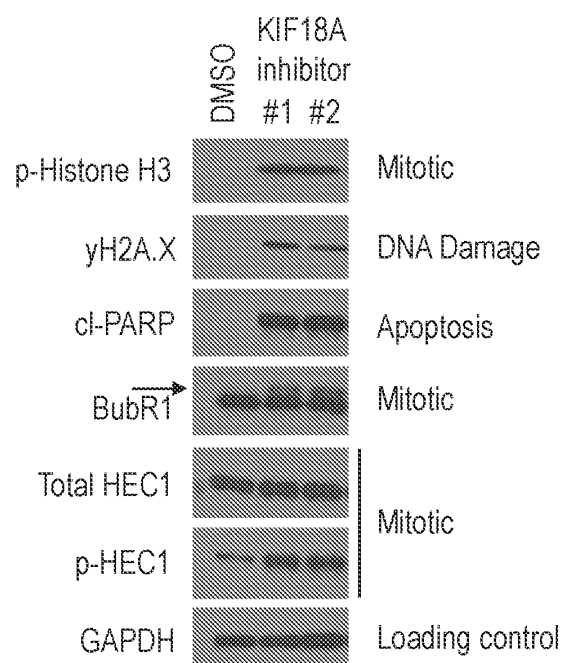


FIG. 10B

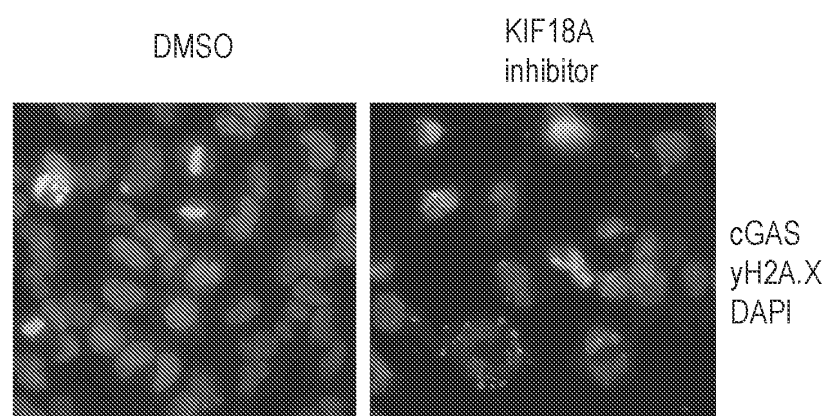


FIG. 11

KIF18A, Centrin-3, DNA(DAPI)

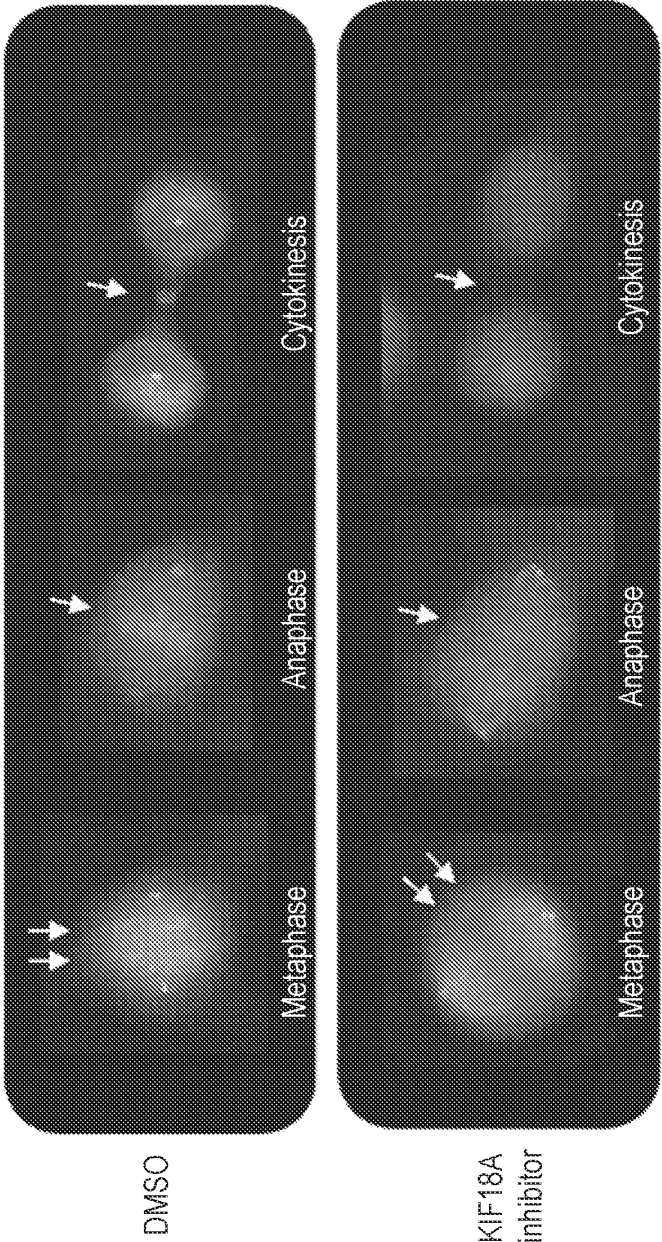
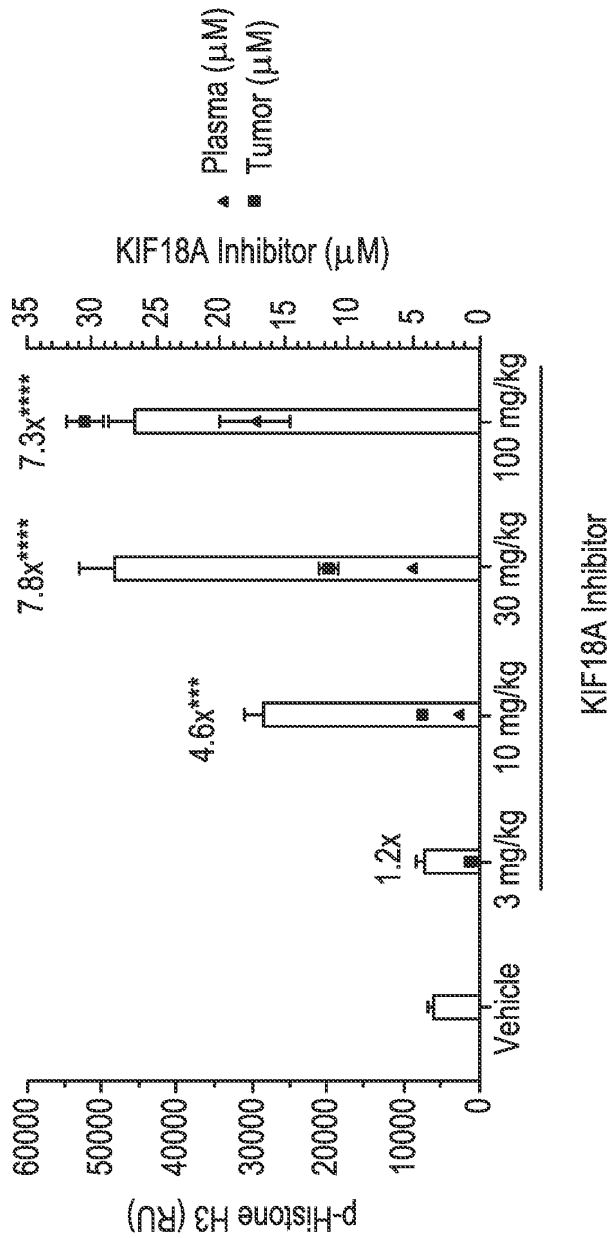


FIG. 12



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FIG. 13A

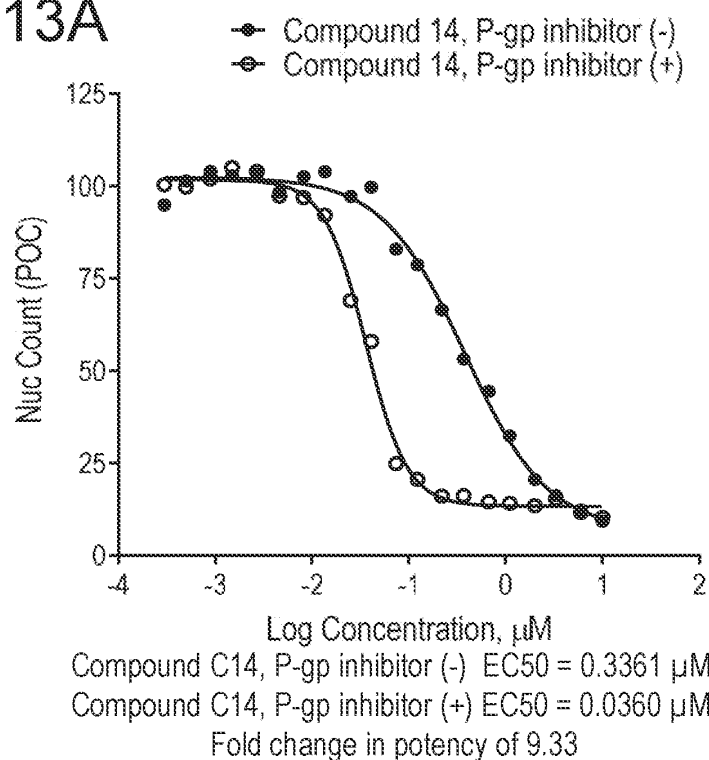


FIG. 13B

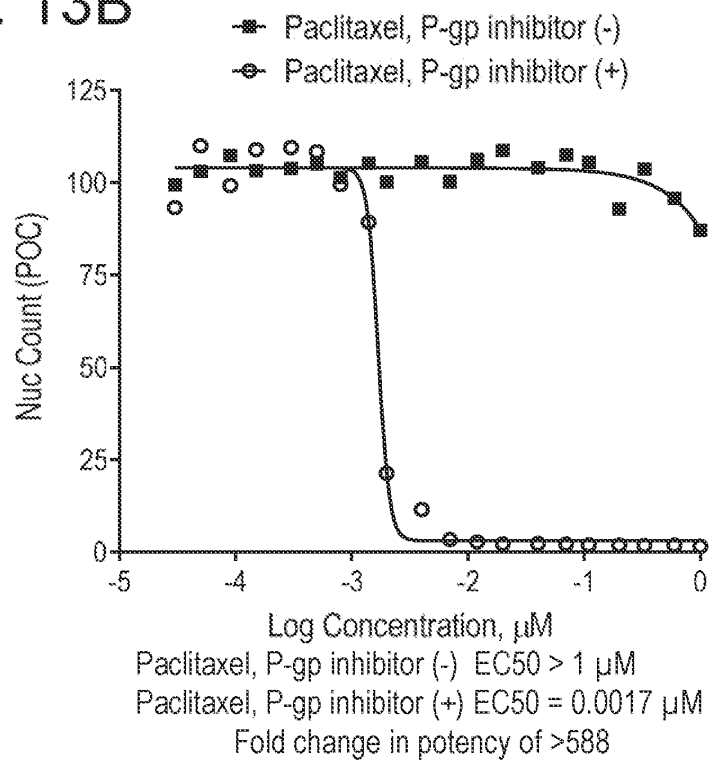


FIG. 14A

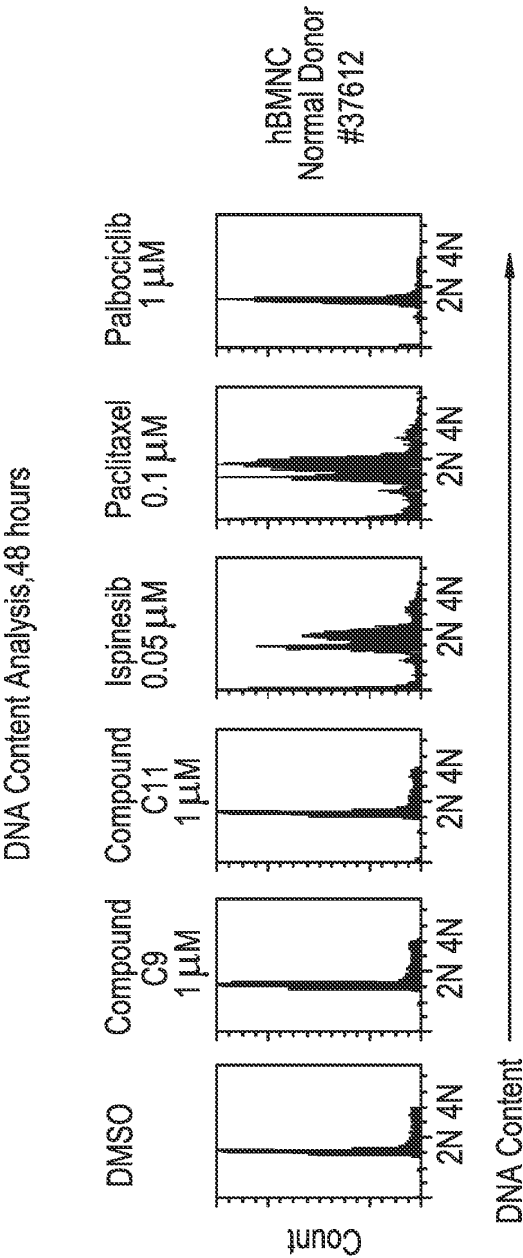


FIG. 14B

BrdU and DNA Content Analysis, 48 hours

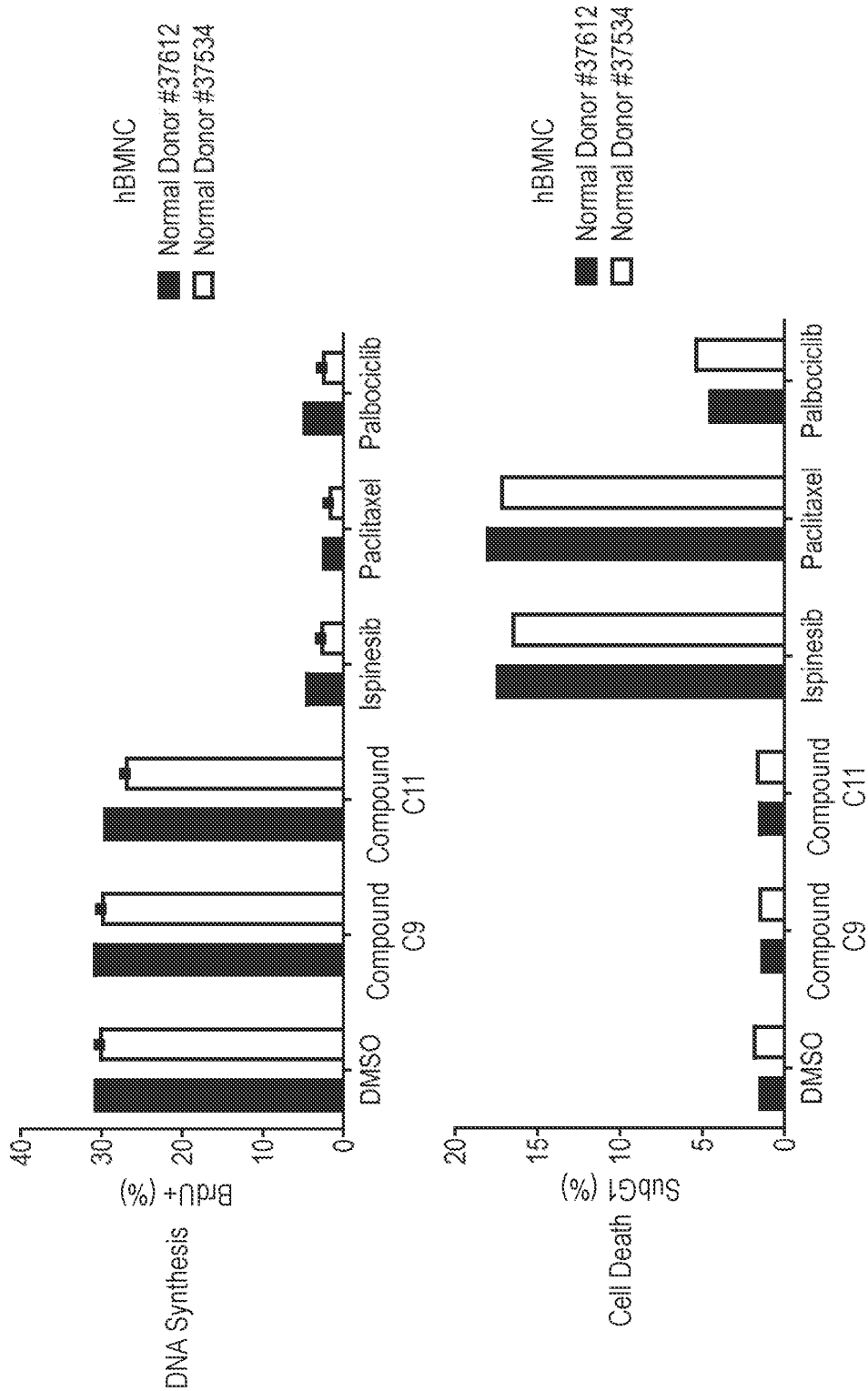


FIG. 14C
4-Day Cell Count Analysis

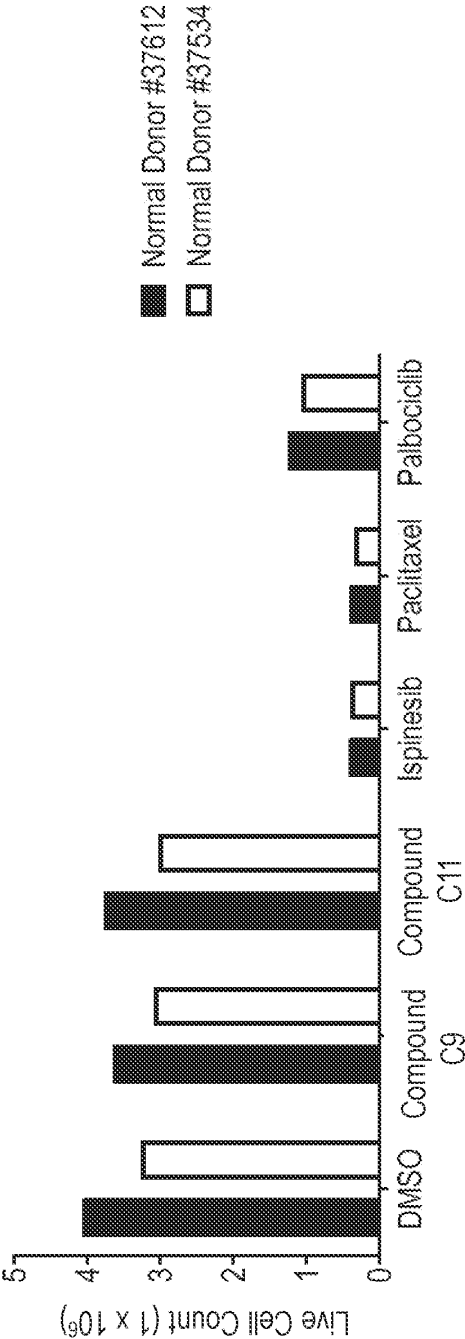
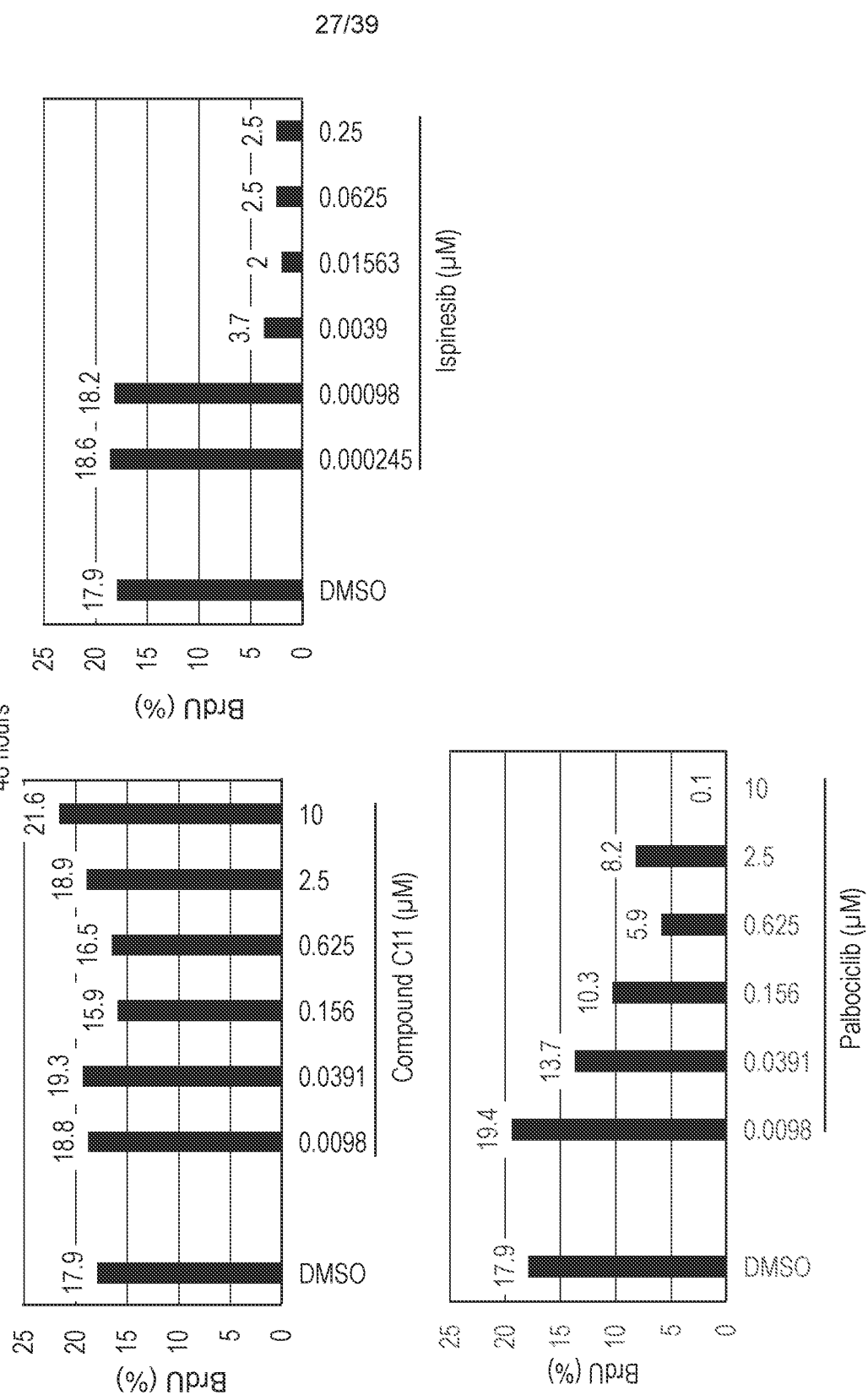


FIG. 14D

hFSF, BrdU Incorporation (%)
48 hours

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FIG. 14E

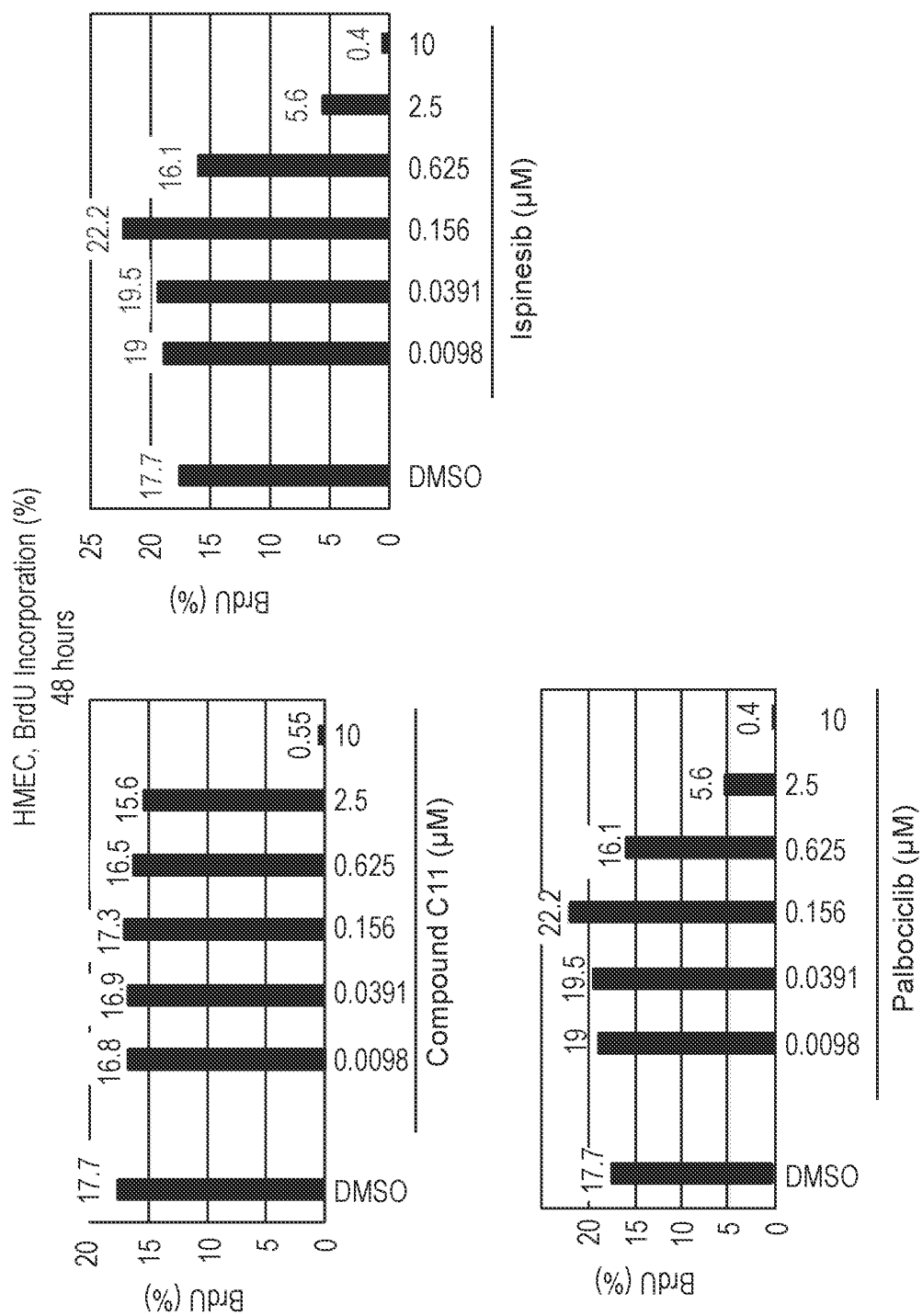


FIG. 15B

BrdU Incorporation (%)

hFSF, 48 hours

FIG. 15A

Total Object Count

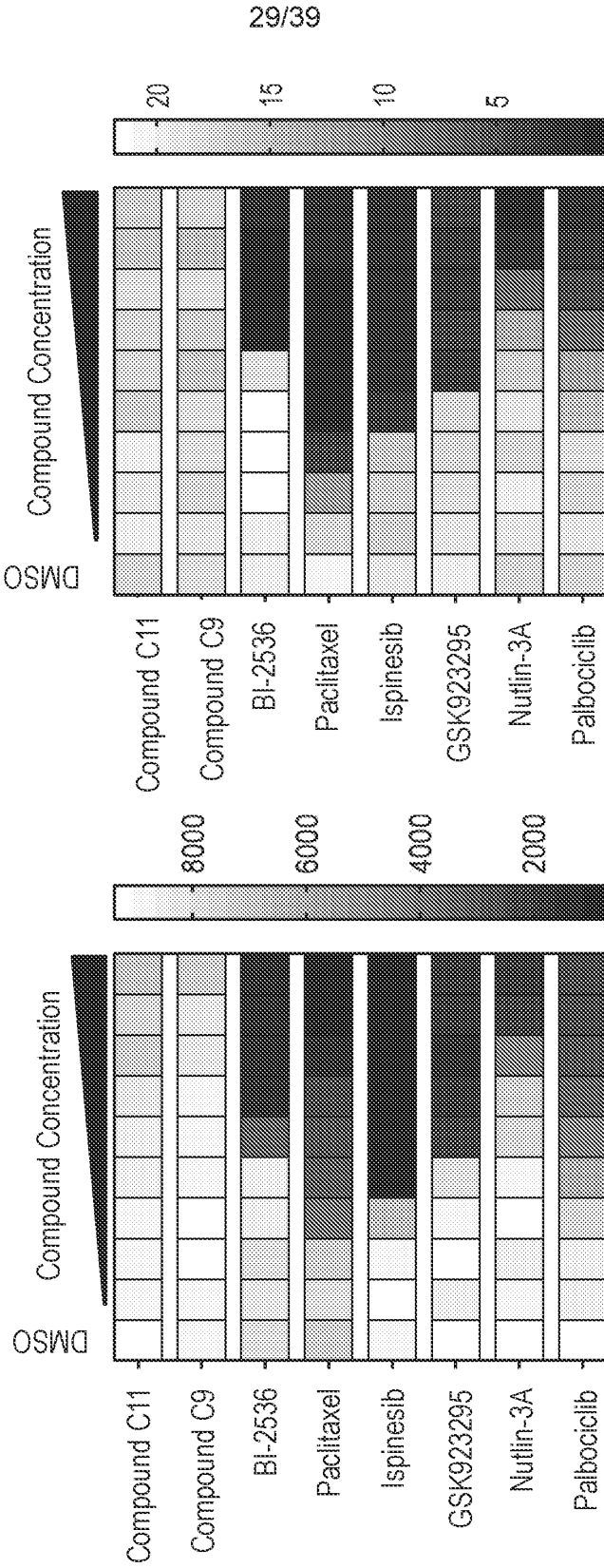


FIG. 15C

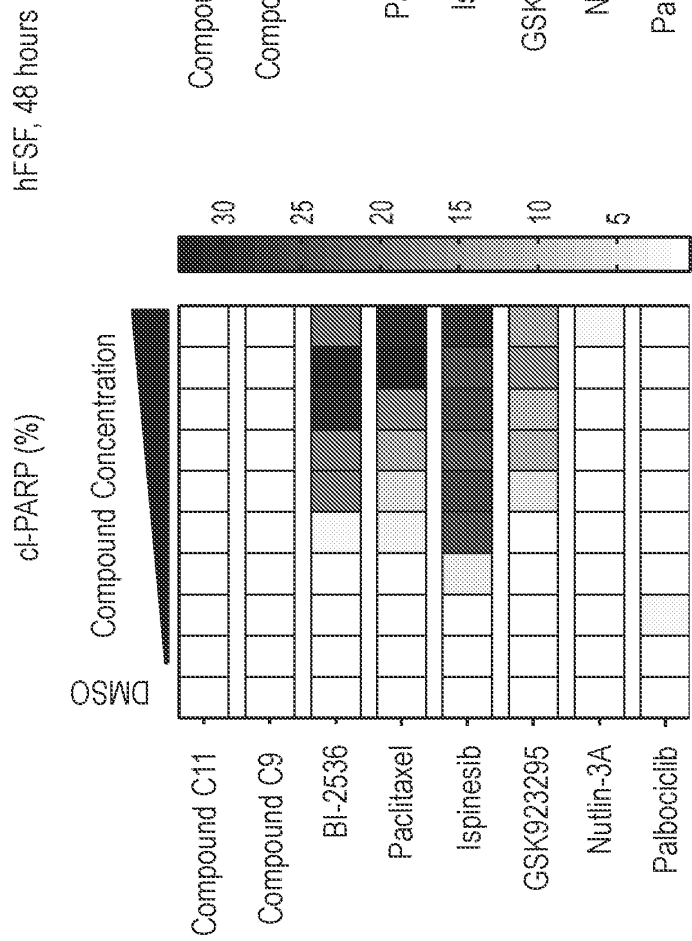
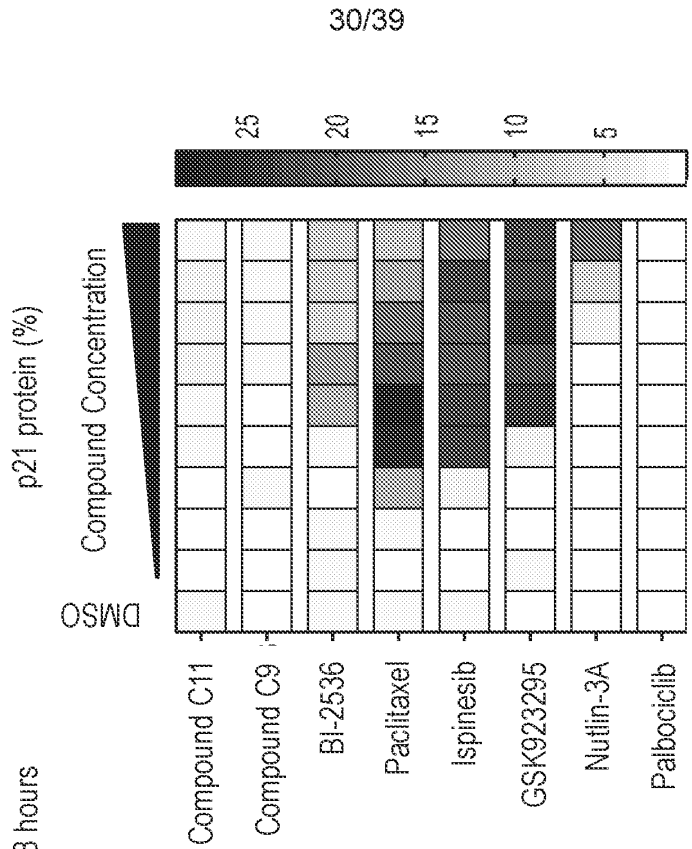


FIG. 15D



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FIG. 15E

hFSF, 48 hours

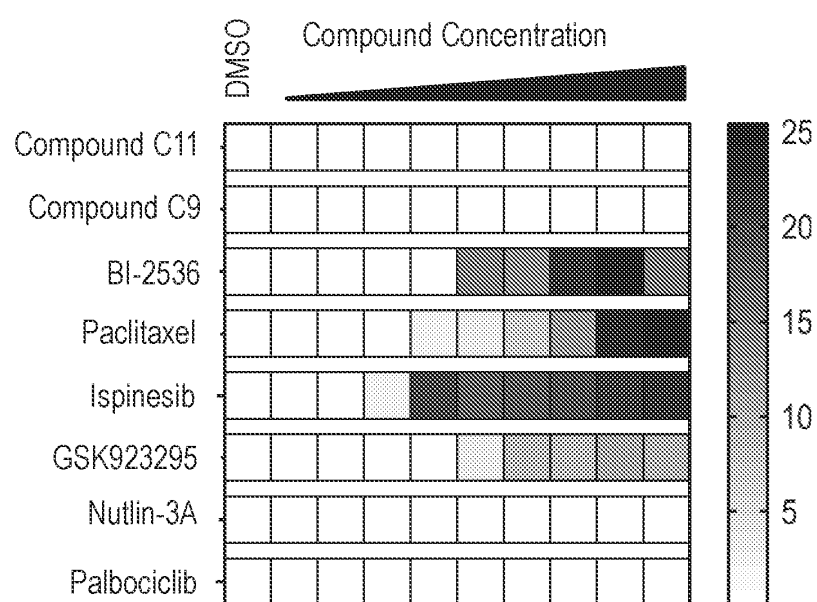
 γ H2X (%)

FIG. 16

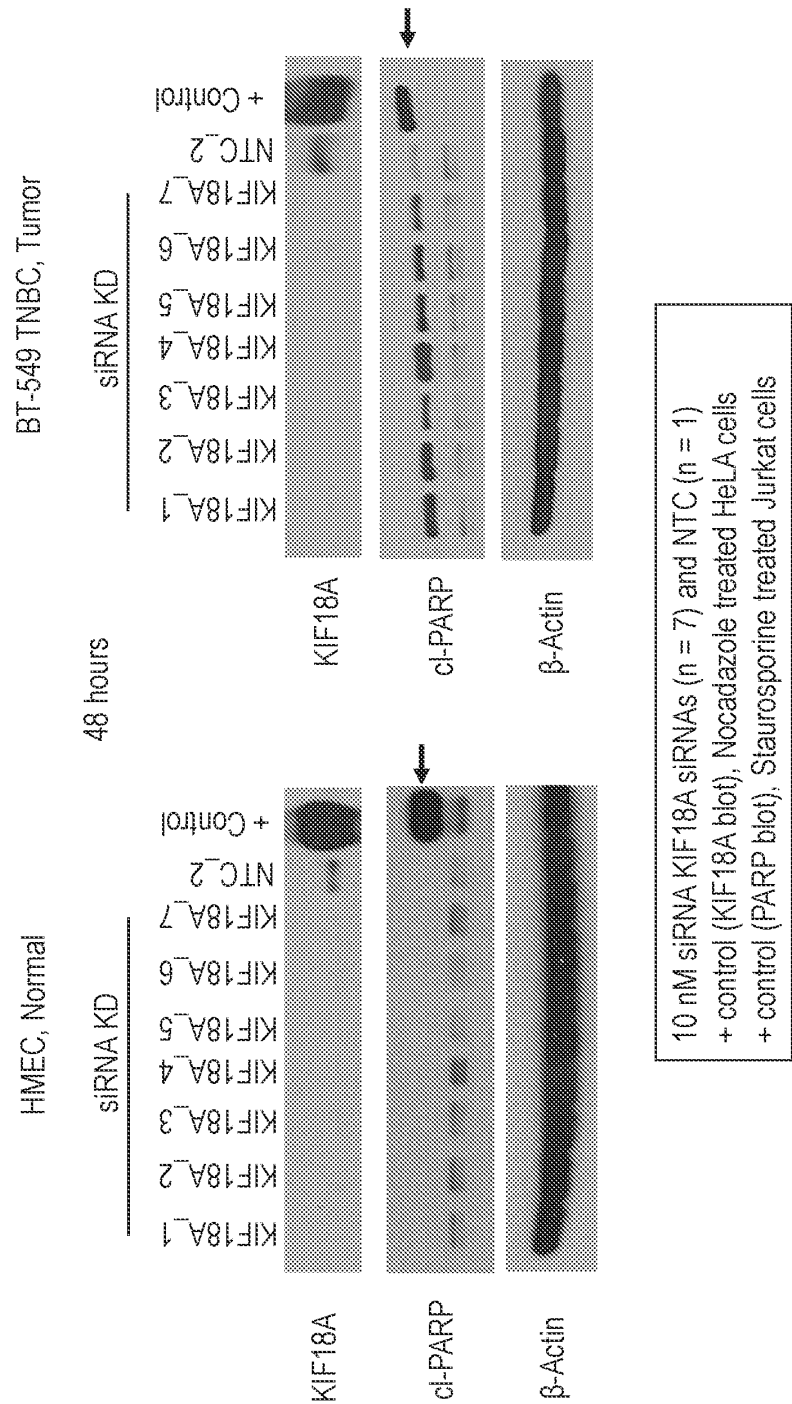


FIG. 17A

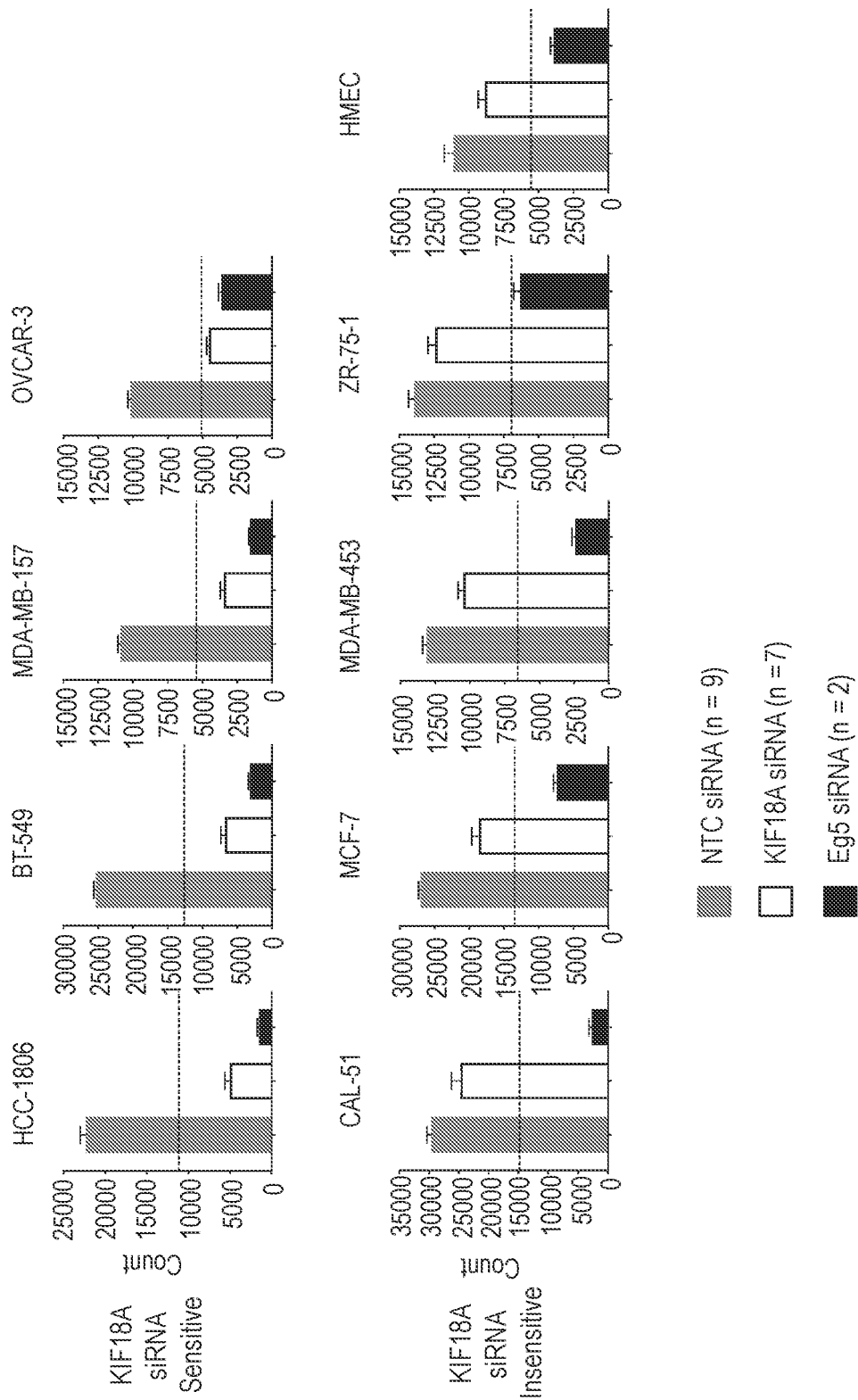


FIG. 17B

Cell Line Information					NTC versus KIF18A siRNAs	NTC versus KIF18A siRNAs
Cell Line	Tissue Origin	Tumor Subtype	TP53 Status	RB1/CCNE1 Status	t-Test, P-value	Count (POC)
HCC-1806	Breast	TNBC	Mutant	CCNE1 Amplified	4.02E-27	*77.5
BT-549	Breast	TNBC	Mutant	RB1-Deficient	2.45E-36	*73.2
MDA-MB-157	Breast	TNBC	Mutant	CCNE1 Amplified	2.36E-31	*68.3
OVCAR-3	Ovary	HGSOC	Mutant	CCNE1 Amplified	5.87E-31	*55.23
MCF-7	Breast	Luminal, ER Positive	Wildtype	RB1-Proficient	1.24E-11	30.9
CAL-51	Breast	TNBC	Wildtype	RB1-Proficient	3.01E-03	16.6
MDA-MB-453	Breast	HER2, ER Negative	Null	RB1-Proficient	3.27E-07	20.3
ZR-75-1	Breast	Luminal, ER Positive	Wildtype	RB1-Proficient	2.48E-02	10.9
HMEC	Breast	-	Wildtype	RB1-Proficient	1.14E-02	20.3
*Cell growth inhibition >50% for KIF18A siRNAs relative to NTC siRNAs						

FIG. 17C

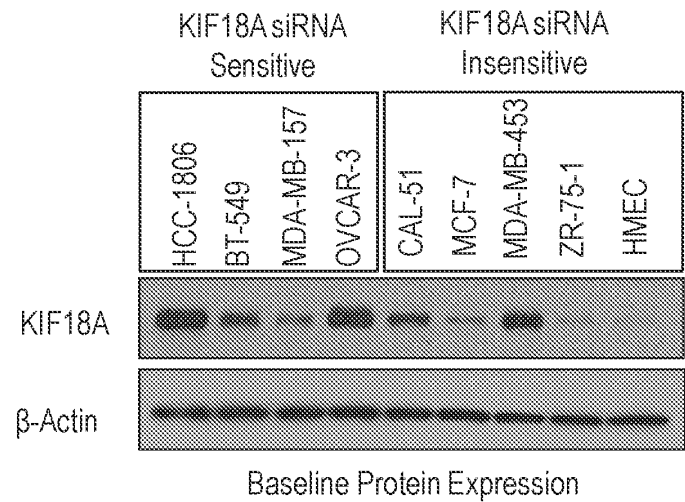
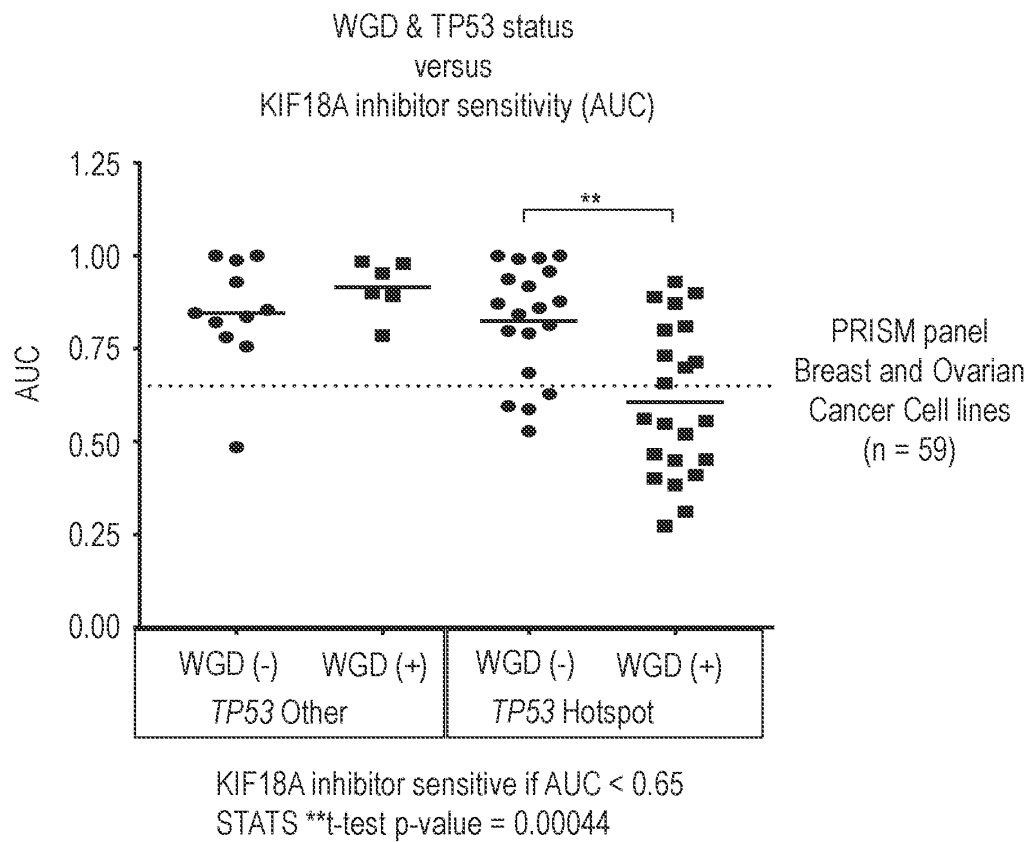


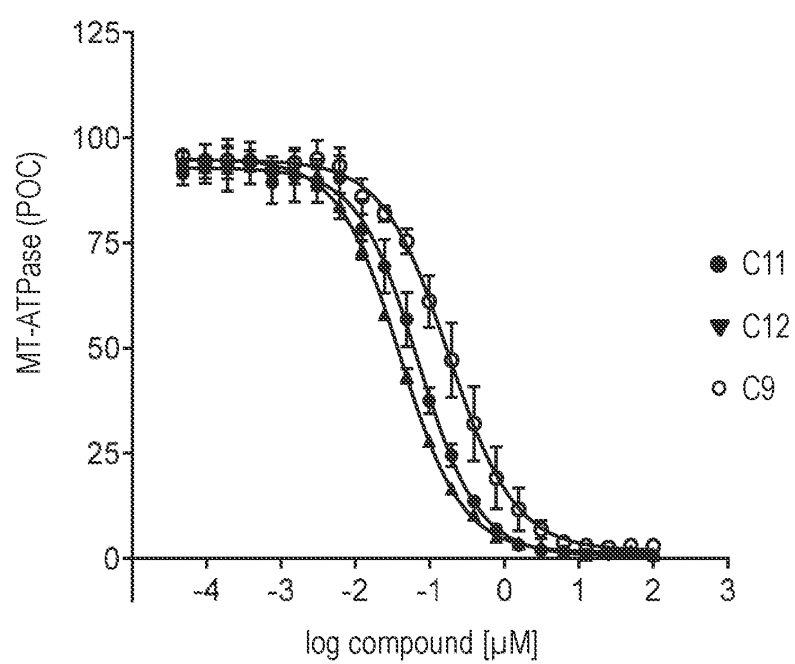
FIG. 18



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FIG. 19A

KIF18A Motor



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FIG. 19B

OVCAR-3 Tumor Xenograft

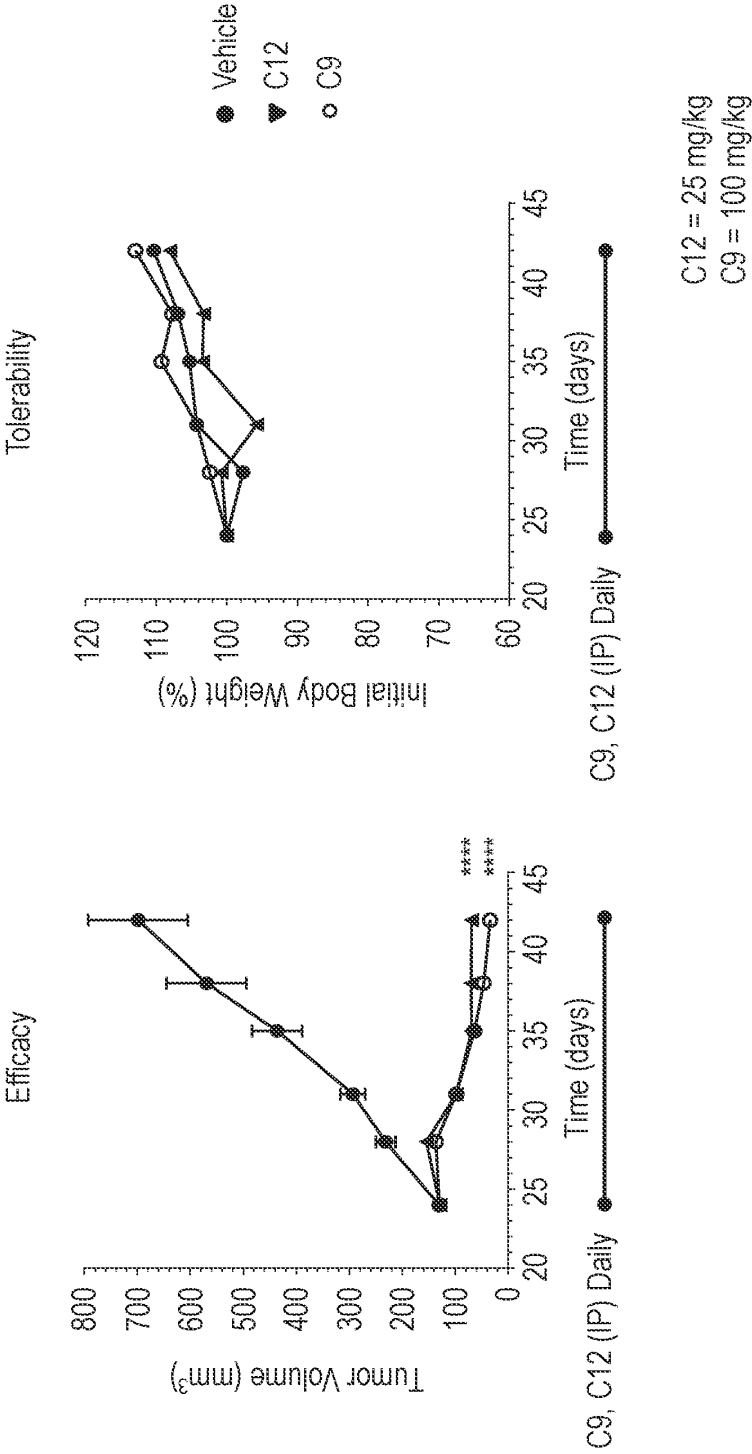


FIG. 19C

CAL-51 Tumor Xenograft

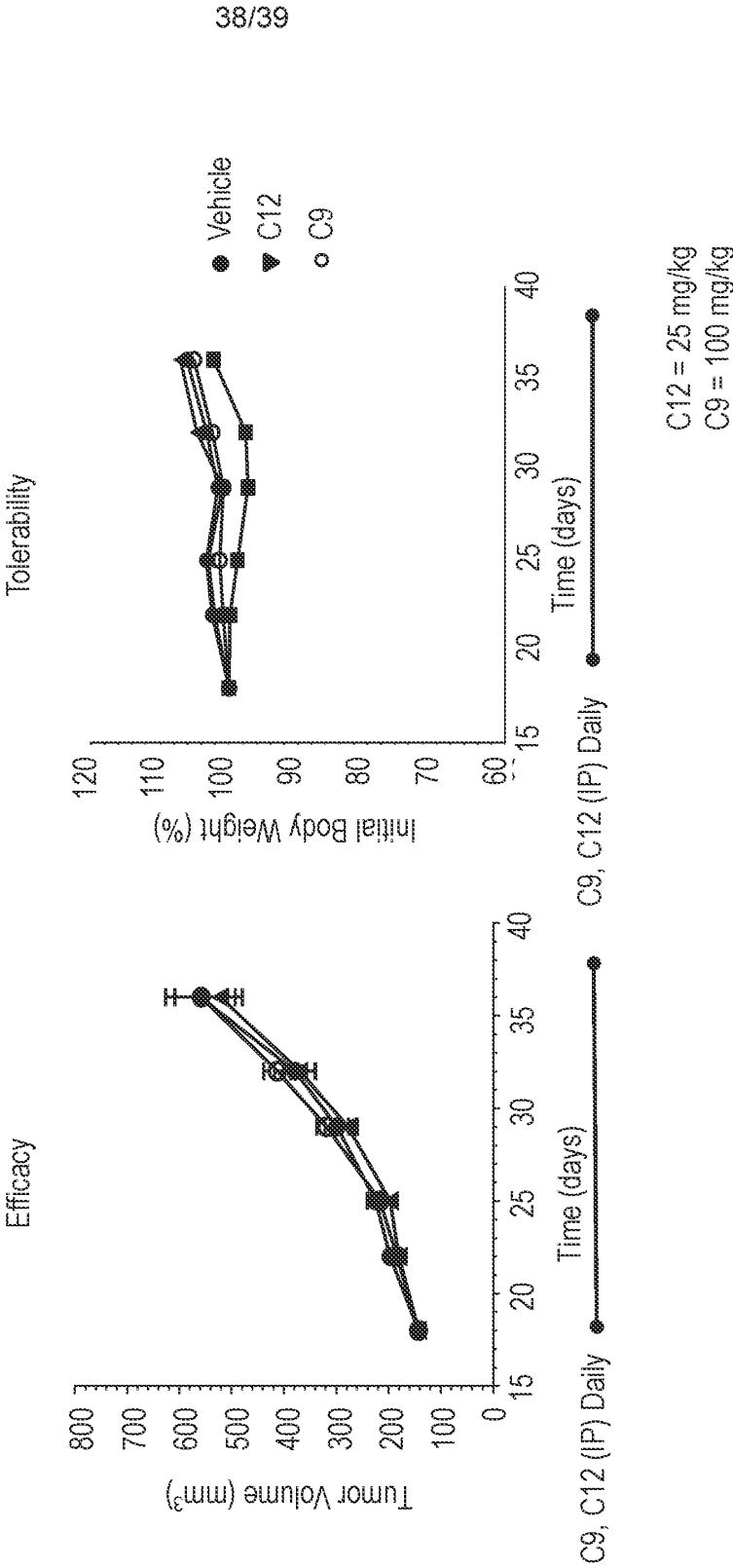


FIG. 19D

OVCAR-8 Tumor Xenograft

