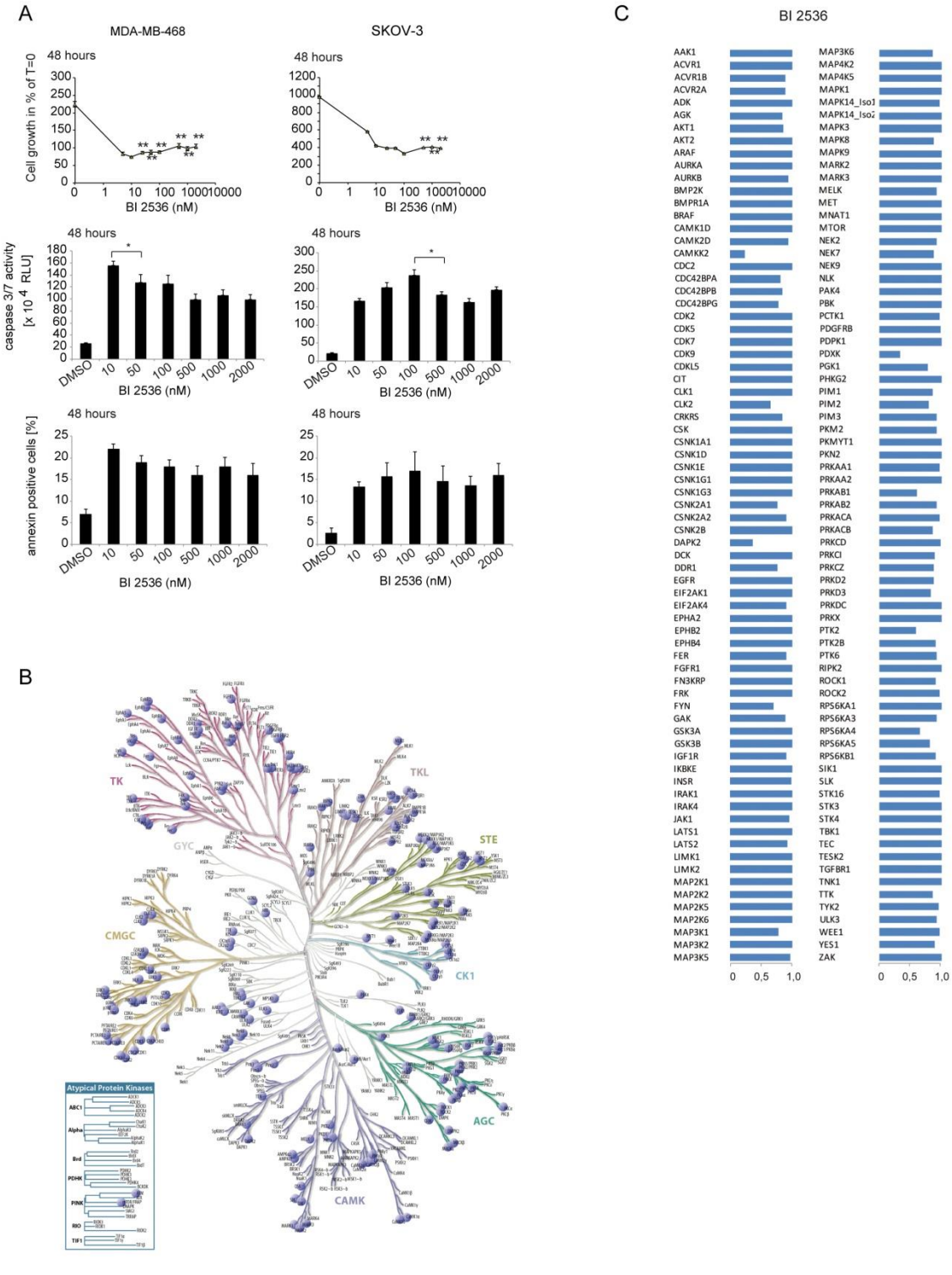
A
MDA-MB-468
SKOV-3
48 hours
Cell growth in % of T=0
BI 2536 (nM)
caspase 3/7 activity [x 10^4 RLU]
annexin positive cells [%]
DMSO
10
50
100
500
1000
2000
B
TK
TKL
STE
GYC
CMGC
CK1
AGC
CAMK
Atypical Protein Kinases
C
BI 2536
AAK1
ACVR1
ACVR1B
ACVR2A
ADK
AGK
AKT1
AKT2
ARAF
AURKA
AURKB
BMP2K
BMPR1A
BRAF
CAMK1D
CAMK2D
CAMKK2
CDC2
CDC42BPA
CDC42BPB
CDC42BPG
CDK2
CDK5
CDK7
CDK9
CDKL5
CIT
CLK1
CLK2
CRKRS
CSK
CSNK1A1
CSNK1D
CSNK1E
CSNK1G1
CSNK1G3
CSNK2A1
CSNK2A2
CSNK2B
DAPK2
DCK
DDR1
EGFR
EIF2AK1
EIF2AK4
EPHA2
EPHB2
EPHB4
FER
FGFR1
FN3KRP
FRK
FYN
GAK
GSK3A
GSK3B
IGF1R
IKBKE
INSR
IRAK1
IRAK4
JAK1
LATS1
LATS2
LIMK1
LIMK2
MAP2K1
MAP2K2
MAP2K5
MAP2K6
MAP3K1
MAP3K2
MAP3K5
MAP3K6
MAP4K2
MAP4K5
MAPK1
MAPK14_Iso1
MAPK14_Iso2
MAPK3
MAPK8
MAPK9
MARK2
MARK3
MELK
MET
MNAT1
MTOR
NEK2
NEK7
NEK9
NLK
PAK4
PBK
PCTK1
PDGFRB
PDPK1
PDXK
PGK1
PHKG2
PIM1
PIM2
PIM3
PKM2
PKMYT1
PKN2
PRKAA1
PRKAA2
PRKAB1
PRKAB2
PRKACA
PRKACB
PRKCD
PRKCI
PRKCZ
PRKD2
PRKD3
PRKDC
PRKX
PTK2
PTK2B
PTK6
RIPK2
ROCK1
ROCK2
RPS6KA1
RPS6KA3
RPS6KA4
RPS6KA5
RPS6KB1
SIK1
SLK
STK16
STK3
STK4
TBK1
TEC
TESK2
TGFBR1
TNK1
TTK
TYK2
ULK3
WEE1
YES1
ZAK
0
0,5
1,0

Figure S1

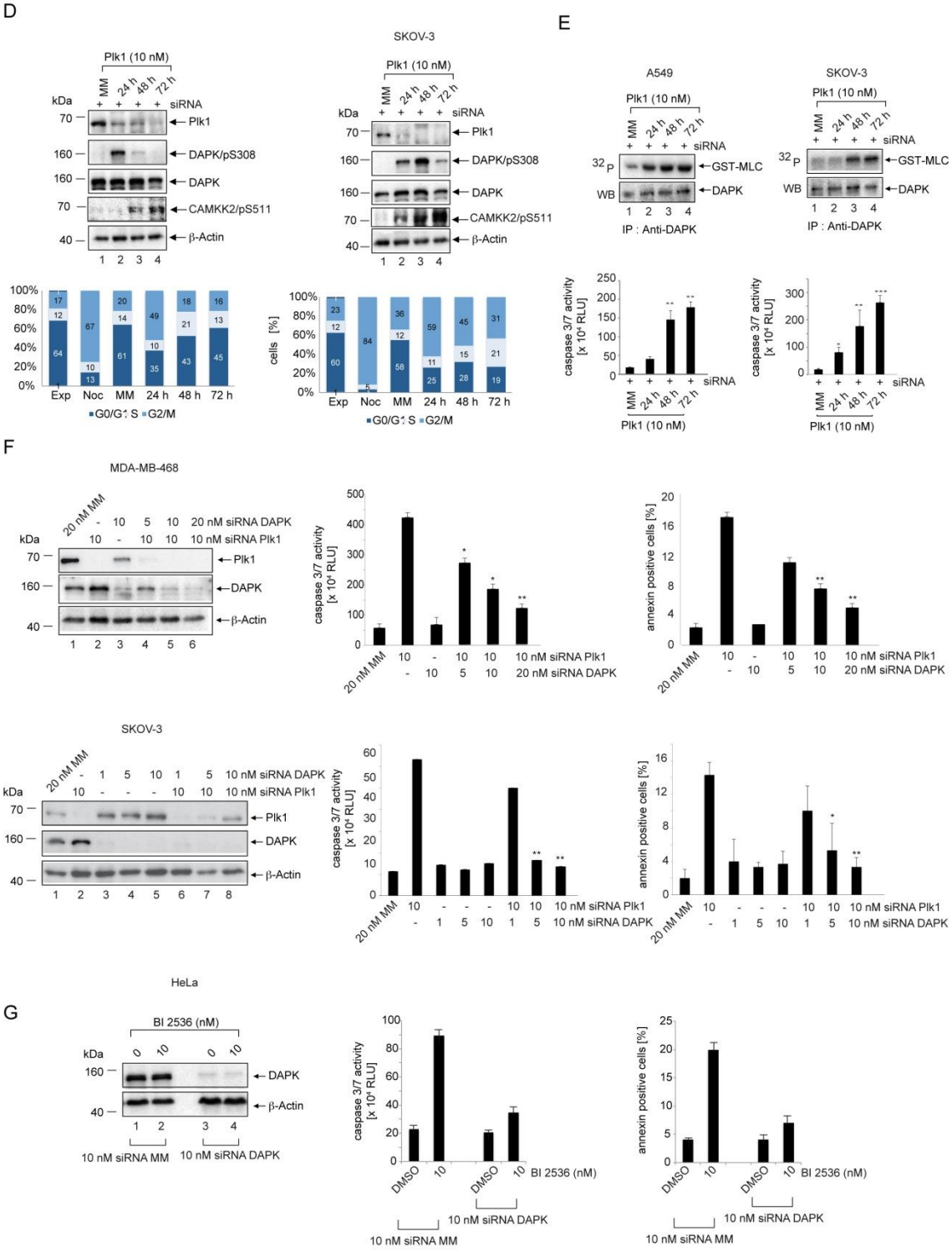
D
SKOV-3
Plk1 (10 nM)
MM
24 h
48 h
72 h
siRNA
kDa
Plk1
DAPK/pS308
DAPK
CAMKK2/pS511
β-Actin
1 2 3 4
100%
80%
60%
40%
20%
0%
cells [%]
Exp
Noc
MM
24 h
48 h
72 h
G0/G1
S
G2/M
E
A549
SKOV-3
32P
GST-MLC
WB
IP : Anti-DAPK
caspase 3/7 activity [x 10⁴ RLU]
F
MDA-MB-468
20 nM MM
20 nM siRNA DAPK
10 nM siRNA Plk1
annexin positive cells [%]
SKOV-3
10 nM siRNA DAPK
G
HeLa
BI 2536 (nM)
DMSO
10 nM siRNA MM
10 nM siRNA DAPK

Figure S1

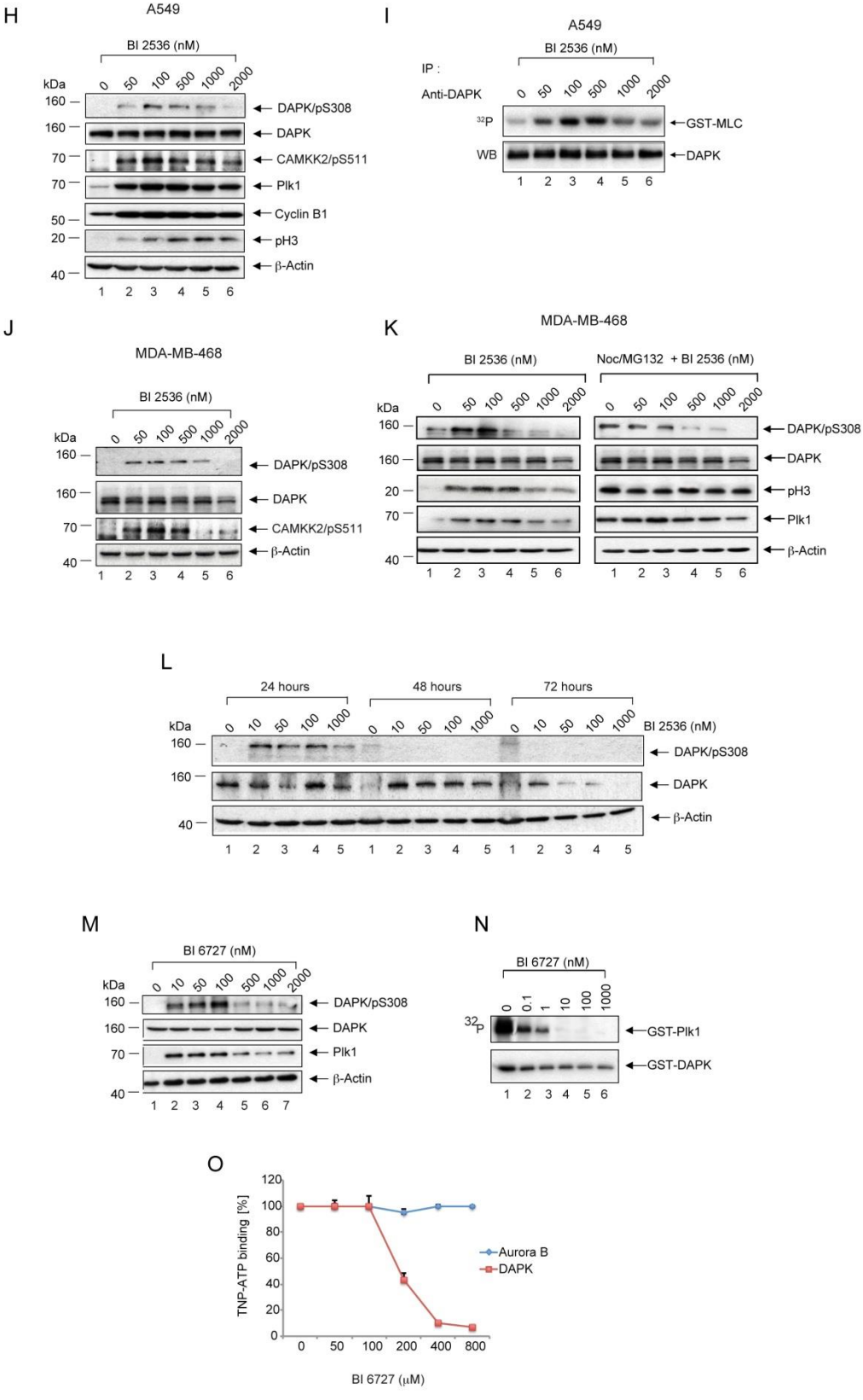
H
A549
BI 2536 (nM)
kDa
0 50 100 500 1000 2000
160
160
70
70
50
20
40
DAPK/pS308
DAPK
CAMKK2/pS511
Plk1
Cyclin B1
pH3
β-Actin
1 2 3 4 5 6
I
A549
BI 2536 (nM)
IP :
Anti-DAPK
0 50 100 500 1000 2000
32P
WB
GST-MLC
DAPK
1 2 3 4 5 6
J
MDA-MB-468
BI 2536 (nM)
kDa
0 50 100 500 1000 2000
160
160
70
40
DAPK/pS308
DAPK
CAMKK2/pS511
β-Actin
1 2 3 4 5 6
K
MDA-MB-468
BI 2536 (nM)
Noc/MG132 + BI 2536 (nM)
kDa
0 50 100 500 1000 2000
160
160
20
70
40
DAPK/pS308
DAPK
pH3
Plk1
β-Actin
1 2 3 4 5 6
L
24 hours
48 hours
72 hours
kDa
0 10 50 100 1000
BI 2536 (nM)
160
160
40
DAPK/pS308
DAPK
β-Actin
1 2 3 4 5
M
BI 6727 (nM)
kDa
0 10 50 100 500 1000 2000
160
160
70
40
DAPK/pS308
DAPK
Plk1
β-Actin
1 2 3 4 5 6 7
N
BI 6727 (nM)
0 0.1 1 10 100 1000
32P
GST-Plk1
GST-DAPK
1 2 3 4 5 6
O
TNP-ATP binding [%]
120
100
80
60
40
20
0
Aurora B
DAPK
0 50 100 200 400 800
BI 6727 (μM)

Figure S1

Supplementary information, Figure S1 (A) Cell proliferation was measured by Cell Titer Blue assay® for MDA-MB-468 and SKOV-3 cells. Concentration-dependent, apoptotic response of MDA-MB-468 and SKOV-3 cells**.** Caspase-3/7 activity was determined in the cell lysates of cells treated at increasing concentrations of BI2536 using the Caspase-Glo® 3/7 Assay (mean values of three independent experiments for each concentration) and 7-AAD was used in conjunction with annexin V staining to discriminate among the viable, apoptotic and necrotic cells using dual parameter FACS analysis (mean values of three independent experiments for each concentration). DMSO is used as the control treatment. **(B)** Proteomic profiling of the Plk1 inhibitor BI2536 by the Kinobead competition assay. Kinobeads coverage of the kinome. The quantitative mass spectrometric analysis of Kinobead competition assays of HeLa cells treated with 250 nM Plk1 inhibitors identified 146 human kinases across all branches of the phylogenetic tree. **(C)** Kinase-binding profiles of BI2536 (blue) across the panel of identified kinases. The length of the bars represents the percentage of activity indicated at the bottom. **(D)** Regulation and activity of DAPK in Plk1-depleted cells. A549 (lung cancer) or SKOV-3 (ovarian cancer) cells were transfected with siRNA Plk1 mismatch (MM) as control or siRNA Plk1 and analyzed at 24 h, 48 h and 72 h after transfection. Lysates were immunoblotted for Plk1, DAPK/pS308, DAPK, CAMKK2/pS511, and β-Actin (upper panel). The cell cycle distribution was analyzed by FACS (lower panel). **(E)** Immunoprecipitated DAPK from the lysates of siRNA-treated cells was subjected to kinase assays using GST-Myosin light chain (MLC) as the substrate (upper panel) and caspase-3/7 activity was determined using the Caspase-Glo® 3/7 Assay (lower panel). **P*<0.05, ***P*<0.01, ****P*<0.001, Student's *t*-test, unpaired and two-tailed. **(F)** Co-depletion of Plk1 and DAPK in cancer cells. MDA-MB-468 or SKOV-3 cells were transfected with siRNA MM as control, siRNA Plk1, siRNA DAPK, or a combination of siRNA DAPK and siRNA Plk1. Lysates were harvested and analyzed by immunoblotting for Plk1, DAPK and β-Actin. For all panels, one image representative of three independent experiments is shown (left panel). Caspase-3/7 activity was determined using the Caspase-Glo® 3/7 Assay (middle panel) and 7-AAD was used in conjunction with annexin V staining to discriminate among the viable, apoptotic and necrotic cells using dual parameter FACS analysis (right panel). **P*<0.05, ***P*<0.01, Student's *t*-test,

unpaired and two-tailed. **(G)** HeLa cells were transfected with siRNA MM as control or siRNA DAPK followed by the treatment with 10 nM BI2536 for 48 h. Lysates were harvested and analyzed by immunoblotting for DAPK and β-Actin. For all panels, one image representative of three independent experiments is shown (left panel). Caspase-3/7 activity was determined using the Caspase-Glo® 3/7 Assay (middle panel) and 7-AAD was used in conjunction with annexin V staining to discriminate among the viable, apoptotic and necrotic cells using dual parameter FACS analysis (right panel). **(H)** Determination of DAPK activities in cancer cells. Lysates of A549 cells treated with increasing concentrations of BI2536 were immunoblotted for DAPK/pS308, DAPK, CAMKK2/pS511, Plk1, Cyclin B1, pH3 and β-Actin. **(I)** The immunoprecipitated DAPK from A549 cell lysates was subjected to kinase assays and monitored for phosphorylation of GST-MLC. **(J)** Lysates of MDA-MB-468 cells treated with increasing concentrations of BI2536 were immunoblotted for DAPK/pS308, DAPK, CAMKK2/pS511, and β-Actin. **(K)** MDA-MB-468 cells enriched in mitosis by nocodazole- or nocodazole/proteasome inhibitor MG132-treatment were incubated with increasing concentrations of BI2536 and lysates were immunoblotted for DAPK/pS308, DAPK, pH3, Plk1 and β-Actin. **(L)** Lysates of HeLa cells treated with increasing concentrations of BI2536 for 24 h, 48 h and 72 h were immunoblotted for DAPK/pS308, DAPK, and β-Actin. **(M)** Lysates of cells treated with increasing concentrations of BI6727 were immunoblotted for DAPK/pS308, DAPK, Plk1, and β-Actin. **(N)** Evaluation of Plk1 and DAPK activities at increasing concentrations of BI6727 using *in vitro* kinase assays. **(O)** TNP-ATP displacement assay for the analysis of BI6727 binding to DAPK and Aurora B.

Supplementary information, Table S1 Percentage of kinase inhibition following treatment with 250 nM BI2536.

	%
CAMKK2	76
CLK2	33
DAPK2	63
MAPK3	22
PDXK	66
PTK2	40
RPS6KA4	34