STable1. Primers sequences

Name	Primer (5'→3')
TM4SF4 Forward	TGTGGGAAGCGATTTGCGA
TM4SF4 Reverse	GATAATCCCCGTCGTGGAAGG
DKK1 Forward	GGGTCTTTGTCGCGATGGTA
DKK1 Reverse	CTGGTACTTATTCCCGCCCG
PIK3R3 Forward	CGATTTCGCAGAGAGGGGAA
PIK3R3 Reverse	AGCCAGACATTCAGGCGTTT
CLDN2 Forward	TATGTCGGTGCCAGCATTGT
CLDN2 Reverse	GCTACCGCCACTCTGTCTTT
AXIN2 Forward	ATTCGGCCACTGTTCAGACG
AXIN2 Reverse	GACAACCAACTCACTGGCCTG
CCNE2 Forward	GGGGGATCAGTCCTTGCATT
CCNE2 Reverse	TCCCCAGCTTAAATCAGGCA
HEXIM1 Forward	AGGACAGTAGGTGGCAATCG
HEXIM1 Reverse	CCCTCTCTCAGGCAGCTAGA
SOX2 Forward	ACCAGCGCATGGACAGTTAC
SOX2 Reverse	CCGTTCATGTAGGTCTGCGA
VAV3 Forward	TACCTTGTGAGGCACAGGAC
VAV3 Reverse	TGCCCAGCACTTTTGGACTT
Actin Forward	AGAGCTACGAGCTGCCTGAC
Actin Reverse	AGCACTGTGTTGGCGTACAG
hsa-miR-103a-3p	AGCAGCATTGTACAGGGCTATGA
hsa-miR-203a-3p	GTGAAATGTTTAGGACCACTAG
hsa-miR-200c-3p	TAATACTGCCGGGTAATGATGGA
hsa-miR-222-3p	AGCTACATCTGGCTACTGGGT
hsa-miR-221-3p	AGCTACATTGTCTGCTGGGTTTC
hsa-miR-30b-5p	TGTAAACATCCTACACTCAGCT
hsa-miR-30c-5p	TGTAAACATCCTACACTCTCAGC
Reverse primer	GCGAGCACAGAATTAATACGAC
U6 Forward	CTCGCTTCGGCAGCACA
U6 Reverse	AACGCTTCACGAATTTGCGT

STable2. The top 70 genes that BPI-9016M downregulated

Gene name	Fold	pvalue	GeneDescription
TM4SF4	4.486	1.08E-82	transmembrane-4-L-six-family-member-4
CLDN2	3.615	2.67E-109	claudin-2
DIO2	3.399	4.44E-19	deiodinase-iodothyronine-type-II
BAAT	3.357	3.55E-17	bile-acid-CoA:amino-acid-N-acyltransferase
TM4SF20	3.218	5.53E-48	Transmembrane-4-L-six-family-member-20
DKK1	3.192	8.04E-129	dickkopf-WNT-signaling-pathway-inhibitor-1
FAM111B	3.140	2.20E-34	family-with-sequence-similarity-111-member-B
DOK3	3.128	5.36E-13	docking-protein-3
E2F8	3.125	8.99E-33	E2F-transcription-factor-8
INSL4	3.063	6.56E-46	insulin-like-4
SFPQ	2.976	3.51E-119	splicing-factor-proline/glutamine-rich
PPP1R10	2.872	2.96E-65	protein-phosphatase-1-regulatory-subunit-10
FBXL19-AS1	2.836	2.85E-13	FBXL19-antisense-RNA-1-(head-to-head)
HNRNPA1P33	2.763	6.69E-26	heterogeneous-nuclear-ribonucleoprotein-A1-pseudogene-33
NPTX1	2.744	2.77E-81	neuronal-pentraxin-I
FGB	2.709	9.45E-26	fibrinogen-beta-chain
RNF43	2.665	3.30E-34	ring-finger-protein-43
TMPO-AS1	2.658	1.17E-14	TMPO-antisense-RNA-1
HSPA2	2.627	1.88E-70	heat-shock-protein-family-A-(Hsp70)-member-2
OLFML3	2.605	9.89E-22	olfactomedin-like-3
SLC12A2	2.598	5.53E-76	solute-carrier-family-12-(sodium/potassium/chloride-transporter)-member-2
RRM2	2.552	5.65E-85	ribonucleotide-reductase-M2
AXIN2	2.544	4.32E-19	axin-2
PCDH9	2.530	1.03E-43	protocadherin-9
RP11-521B24.3	2.478	2.73E-08	NA
RP11-311F12.2	2.469	1.21E-06	NA
C6orf99	2.468	4.67E-08	chromosome-6-open-reading-frame-99
ATF5	2.434	1.09E-29	activating-transcription-factor-5
RP11-110I1.12	2.418	5.11E-07	NA
CLSPN	2.410	5.10E-28	claspin

MMP24	2.391	7.24E-57	matrix-metallopeptidase-24
RP11-81N13.1	2.364	9.40E-09	SUCLG2-antisense-RNA-1-(head-to-head)
PIK3R3	2.329	4.40E-09	phosphoinositide-3-kinase-regulatory-subunit-3
PDE3A	2.308	3.42E-21	phosphodiesterase-3A
H2AFX	2.288	4.35E-43	H2A-histone-family-member-X
TMEM2	2.252	1.14E-56	transmembrane-protein-2
C10orf91	2.247	3.91E-10	chromosome-10-open-reading-frame-91
CSPG5	2.240	1.08E-08	chondroitin-sulfate-proteoglycan-5
CCNE2	2.220	1.69E-07	cyclin-E2
KLHL4	2.217	2.32E-07	kelch-like-family-member-4
KCNJ16	2.215	1.49E-05	potassium-channel-inwardly-rectifying-subfamily-J-member-16
EHF	2.206	1.53E-17	ets-homologous-factor
C1QTNF6	2.204	3.56E-40	C1q-and-tumor-necrosis-factor-related-protein-6
TPPP3	2.203	9.01E-18	tubulin-polymerization-promoting-protein-family-member-3
FUS	2.184	1.97E-59	FUS-RNA-binding-protein
ADAMTS9	2.182	2.50E-12	ADAM-metallopeptidase-with-thrombospondin-type-1-motif-9
HEXIM1	2.174	3.56E-64	hexamethylene-bis-acetamide-inducible-1
SNHG14	2.173	4.04E-27	small-nucleolar-RNA-host-gene-14
STS	2.170	7.58E-12	steroid-sulfatase-(microsomal)-isozyme-S
HAS2	2.166	2.72E-08	hyaluronan-synthase-2
IL1R1	2.155	3.73E-31	interleukin-1-receptor-type-I
ACTRT3	2.143	5.13E-06	actin-related-protein-T3
RP11-67K19.3	2.123	1.96E-05	NA
DDX46	2.106	2.76E-51	DEAD-(Asp-Glu-Ala-Asp)-box-polypeptide-46
APOH	2.099	1.54E-09	apolipoprotein-H
SOX2	2.097	2.93E-09	SRY-box-2
MEPCE	2.090	5.95E-43	methylphosphate-capping-enzyme
UHRF1	2.090	8.63E-51	NA
SGK1	2.083	1.32E-24	serum/glucocorticoid-regulated-kinase-1
ANLN	2.077	9.71E-41	anillin-actin-binding-protein
VAV3	2.075	5.84E-19	vav-guanine-nucleotide-exchange-factor-3
ZC3H4	2.073	2.82E-46	zinc-finger-CCCH-type-containing-4
ZNF326	2.073	2.74E-30	zinc-finger-protein-326
HAVCR1	2.070	4.32E-23	hepatitis-A-virus-cellular-receptor-1

ARSB	2.070	2.45E-24	arylsulfatase-B
SPC25	2.064	7.45E-21	SPC25-NDC80-kinetochore-complex-component
BEND3	2.055	1.95E-14	BEN-domain-containing-3
CDKN2C	2.052	2.22E-27	Cyclin dependent-kinase-inhibitor-2C-(p18-inhibits-CDK4)
FGA	2.043	3.64E-20	fibrinogen-alpha-chain
LANCL2	2.036	2.42E-17	LanC-lantibiotic-synthetase-component-C-like-2-(bacterial)

Supplemental method

Cell cycle assay

Cells were fixed in 70% cold ethanol overnight at 4 °C after treatment with BPI-9016M for 24h. Cells were stained with 50 μg/mL propidium iodide (BD Biosciences) at room temperature for 15 min in the dark, and the cell cycle distribution was assessed using a BD Accuri C6 flow cytometer. Data were analyzed by ModFit 3.0 software (BD Biosciences).

Wound healing assay

After the cells were attached in medium containing 1% serum, cell spreading was measured with a wound-healing assay in which the distance of the wound was monitored at 12, 24, and 36h. The wound healing rate was calculated.

Transwell assay

Cells were seeded in transwell chambers (Cat NO. 3422, Corning Costar, Cambridge, MA) to evaluate migration or invasion with matrigel (Cat NO. 356234, BD Biosciences, San Jose, California, USA). After 6-12h incubation, cells that passed through the membrane were fixed and stained with crystal violet. 5 random microscopic fields were used for counting.

RNA sequencing and bioinformatics analysis

Total RNA of A549 cells treated with BPI-9016M was extracted using RNeasy Mini Kit (QIAGEN) according to the manufacturer's protocol. mRNA sequences were conducted using NEBNext® Ultra™ RNA Library Prep Kit from Illumina® (NEB, USA) following the manufacturer's protocols. According to the KEGG

database, the significant signals of different genes were identified using the pathway analysis according to the KEGG database.

Supplemental results

Cell cycle distribution, migration and invasion analyses

BPI-9016M induced accumulation of more tumor cells in the G1 phase (Figure S1A). We performed transwell assay in the several lung adenocarcinoma cell lines including H1299, A549, H1975, PC-9, H1650 and HCC827. A549 and H1299 showed enhanced abilities of migration and invasion than other cells (Figure S1B).

BPI-9016M inhibits the ability of cell spreading

Slower spreading ability was demonstrated in the A549 and H1299 cells treated with BPI-9016M for 12, 24, and 36 h **(Figure S2A)**. BPI-9016M inhibited the enhanced migration and invasion by HGF stimulation in both cell lines **(Figure S2B)**.

Signaling pathways enrichment in BPI-9016M-treated A549 cells

Based on BPI-9016M treatment, numerous signaling pathways including cell cycle, MAPK, Wnt, TGF-beta, adherens junction, p53, mTOR, and VEGF were altered in A549 cells by RNA sequencing (**Figure S3**).

BPI-9016M/miR203/DKK1 signals influence cell spreading, migration and invasion

Figure S4A showed that knock-down of DKK1 inhibited spreading of A549 and H1299 cells. DKK1 overexpression promoted cell spreading and BPI-9016M treatment could partially reverse this effect **(Figure S4B)**. In addition, cell spreading, migration and invasion were enhanced with miR203 inhibitor and the effect could be weakened by BPI-9016M **(Figure S5A-B)**.

Supplemental figure legends

Figure S1. （**A**）The representative images of the cell cycle distribution in A549 and H1299 cells with BPI-9016M treatment. （**B**）The representative photographs (left panel) and histogram (right panel) of Trans-well assays in the several lung adenocarcinoma cell lines.

Figure S2. (A) The representative photographs of wound-healing assay in the A549 and H1299 cells treated with BPI-9016M or vehicle for 12, 24, and 36 hours.（**B**）The

representative photographs of wound-healing assay in the A549 and H1299 cells treated with BPI-9016M, HGF or combination of these two agents for 12, 24, and 36 hours.

Figure S3. Heatmap of the top representative down-regulated genes associated with metastasis in A549 cells with vehicle (control) or BPI-9016M treatment.

Figure S4.（**A**）The representative photographs of wound-healing assay in the A549 and H1299 cells with knock-down of DKK1 for 12, 24, and 36 hours.（**B**）The representative photographs of wound-healing assay in the DKK1 knock-downed A549 and H1299 cells with and without BPI-9016M treatment for 12, 24, and 36 hours.

Figure S5.（**A**）The representative photographs of wound-healing assay in the A549 and H1299 cells treated with miR203 inhibitor for 12, 24, and 36 hours.（**B**）The representative photographs of Trans-well assay in the miR203 knock-downed A549 and H1299 cells with and without BPI-9016M treatment.

Supplemental figures

Figure S1

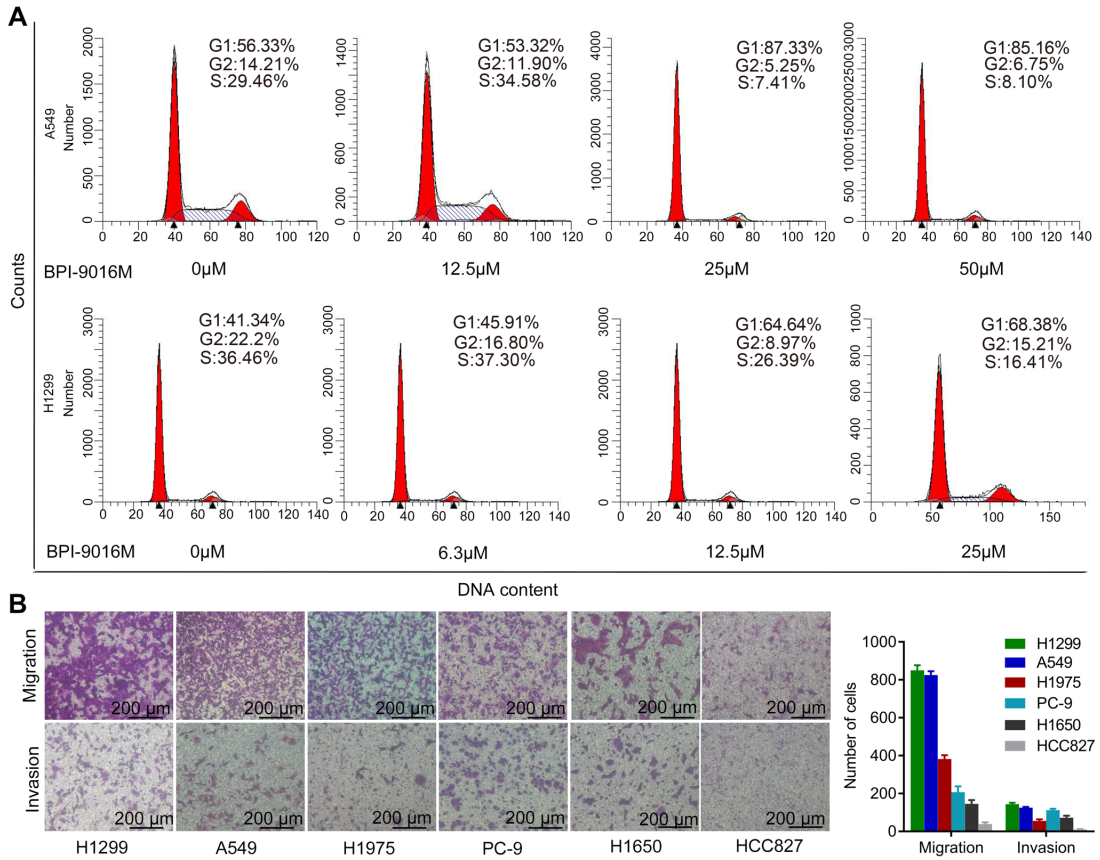


Figure S2

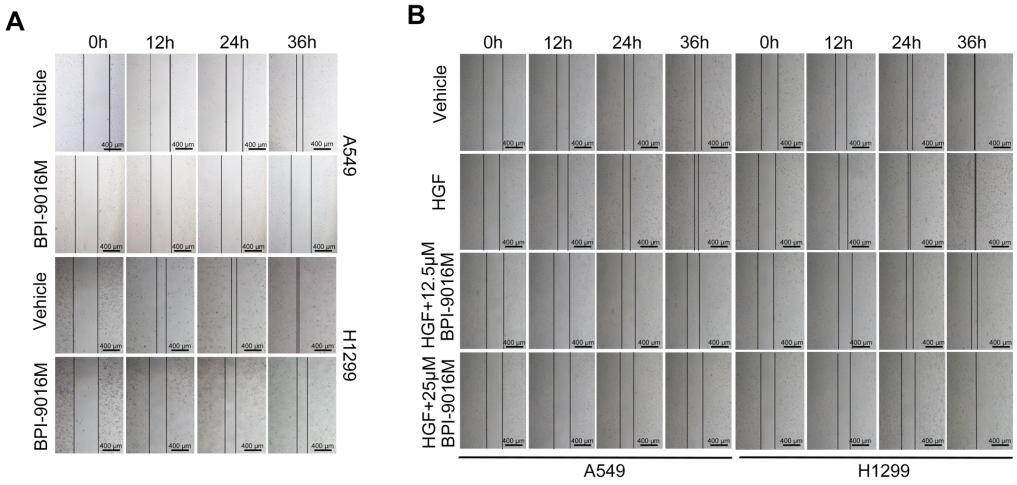

A
0h
12h
24h
36h
Vehicle
BPI-9016M
Vehicle
BPI-9016M
A549
H1299
400 μm
B
0h
12h
24h
36h
0h
12h
24h
36h
Vehicle
HGF
HGF+12.5μM BPI-9016M
HGF+25μM BPI-9016M
A549
H1299
400 μm

Figure S3

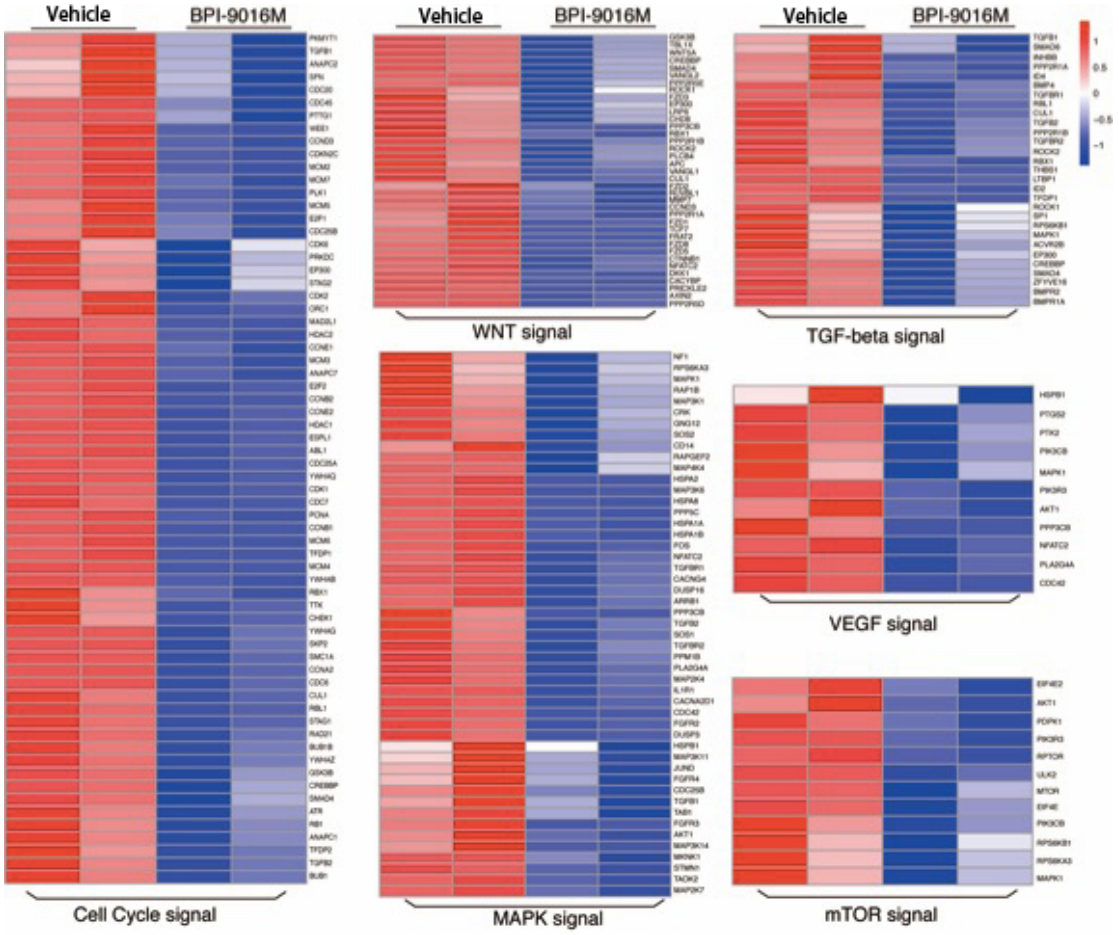
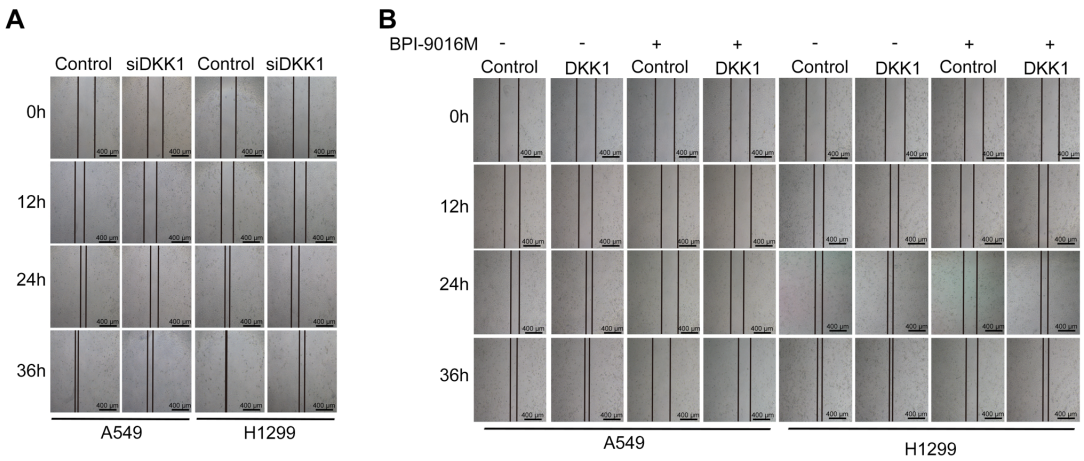

Vehicle
BPI-9016M
Cell Cycle signal
WNT signal
MAPK signal
TGF-beta signal
VEGF signal
mTOR signal

Figure S4


A
Control
siDKK1
Control
siDKK1
0h
12h
24h
36h
A549
H1299
B
BPI-9016M
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-
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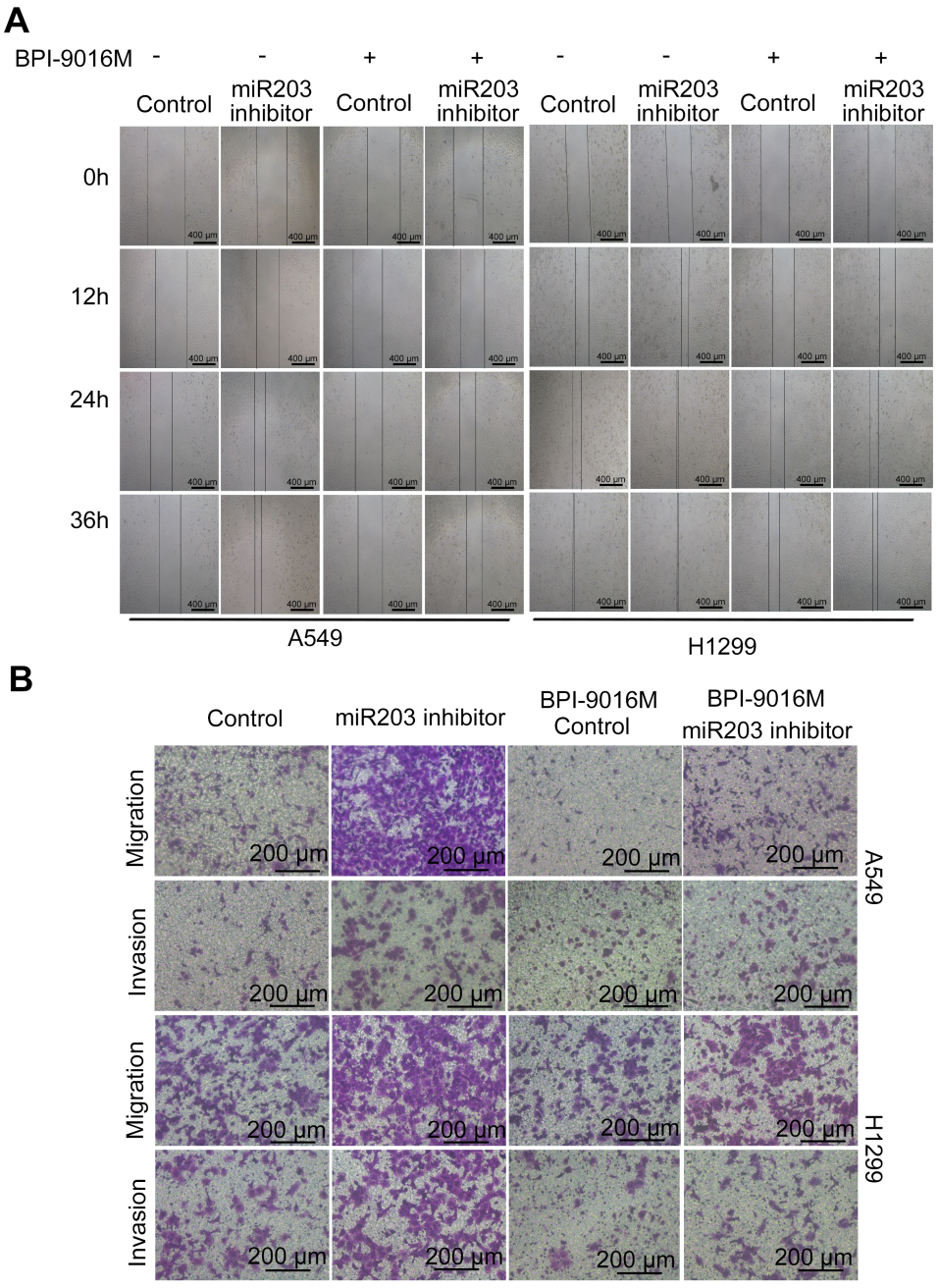
0h
12h
24h
36h
A549
H1299

Figure 5S


A
BPI-9016M - - + + - - + +
Control
miR203 inhibitor
Control
miR203 inhibitor
Control
miR203 inhibitor
Control
miR203 inhibitor
0h
12h
24h
36h
400 μm
A549
H1299
B
Control
miR203 inhibitor
BPI-9016M Control
BPI-9016M miR203 inhibitor
Migration
Invasion
Migration
Invasion
200 μm
A549
H1299