

## Supplementary Data 1

**Table 1 : Primary antibodies used for Western blot analyses and immunohistochemistry**

<b>Antibodies for Western blot analyses</b>				
<b>Targets (Manufacturer)</b>	<b>Phosphorylated residues</b>	<b>Species</b>	<b>Dilution used</b>	<b>References</b>
<b>P-mTOR(Cell Signaling)</b>	Ser2448	Rabbit	1/1000	2971
<b>mTOR(Cell Signaling)</b>		Rabbit	1/1000	2972
<b>P-AKT(Cell Signaling)</b>	Thr 308	Rabbit	1/1000	2965
<b>P-AKT(Cell Signaling)</b>	Ser 473	Rabbit	1/1000	9271
<b>AKT(Cell Signaling)</b>		Rabbit	1/1000	9272
<b>PARP (Cell Signaling)</b>		Rabbit	1/1000	9542

<b>Antibodies for immunohistochemistry</b>				
<b>KI67 (Abcam)</b>		Rabbit	1/50	ab16667
<b>CD31 (Abcam)</b>		Rabbit	1/100	ab75769
<b>CD146 (Abcam)</b>		Rabbit	1/200	ab175769
<b>Osterix (Abcam)</b>		Rabbit	1/800	Ab22552

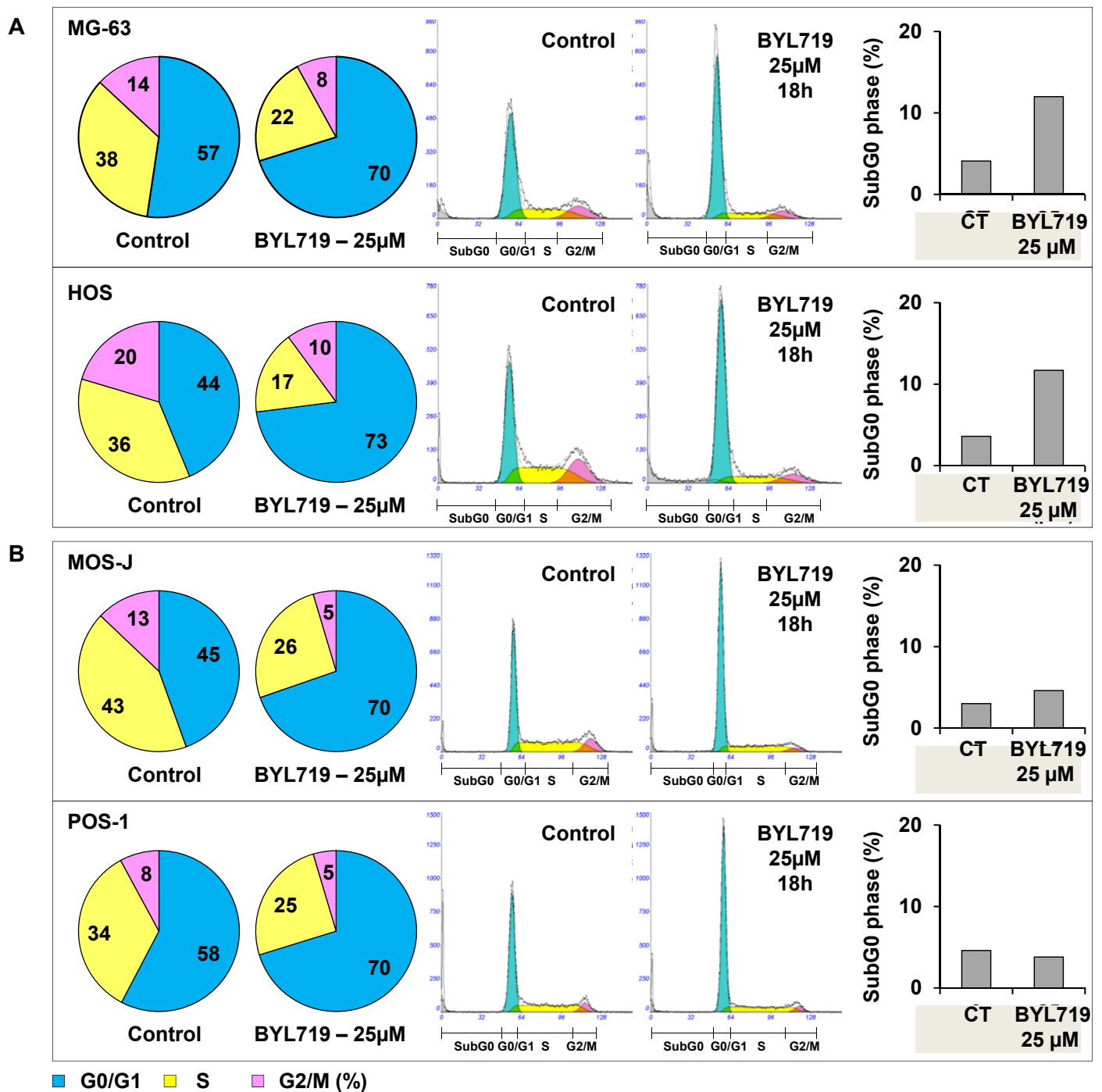
## Supplementary data 2

**Table 2: Primer sequences for PCR experiments**

<b>Name</b>	<b>Primer sequence</b>
<i>NFATc1</i>	(for) 5'-GGTCTTCGGGAGAGGAGAAA (rev) 5'-TGACGTTGGAGGATGCATAG
<i>CATHK</i>	(for) 5'-GCCAGACAACAGATTTCCATC (rev) 5'-CAGAGCAAAGCTCACCACAG
<i>MMP9</i>	(for) 5'-GAACCAATCTCACCGACAGG (rev) 5'-GCCCCAGAGATTTGACTC
<i>TRAP</i>	(for) 5'-AAGACTCACTGGGTGGCTTTG (rev) 5'-GGCAGTCATGGGAGTTCAGG
<i>RUNX2</i>	(for) 5'-GCCTAGGCGCATTTCAGA (rev) 5'-GCTCTTCTTACTGAGAGTGGAAGG
<i>ALP</i>	(for) 5'-AACACCACCCAGGGGAAC (rev) 5'-GGTCACAATGCCACAGATT
<i>GAPDH</i>	(for) 5'-TGGGTGTGAACCATGAGAAGTATG (rev) 5'-GGTGCAGGAGGCATTGCT
<i>ACTB</i>	(for) 5'-CCAACCGCGAGAAGATGA (rev) 5'-CCAGAGGCGTACAGGGATAG

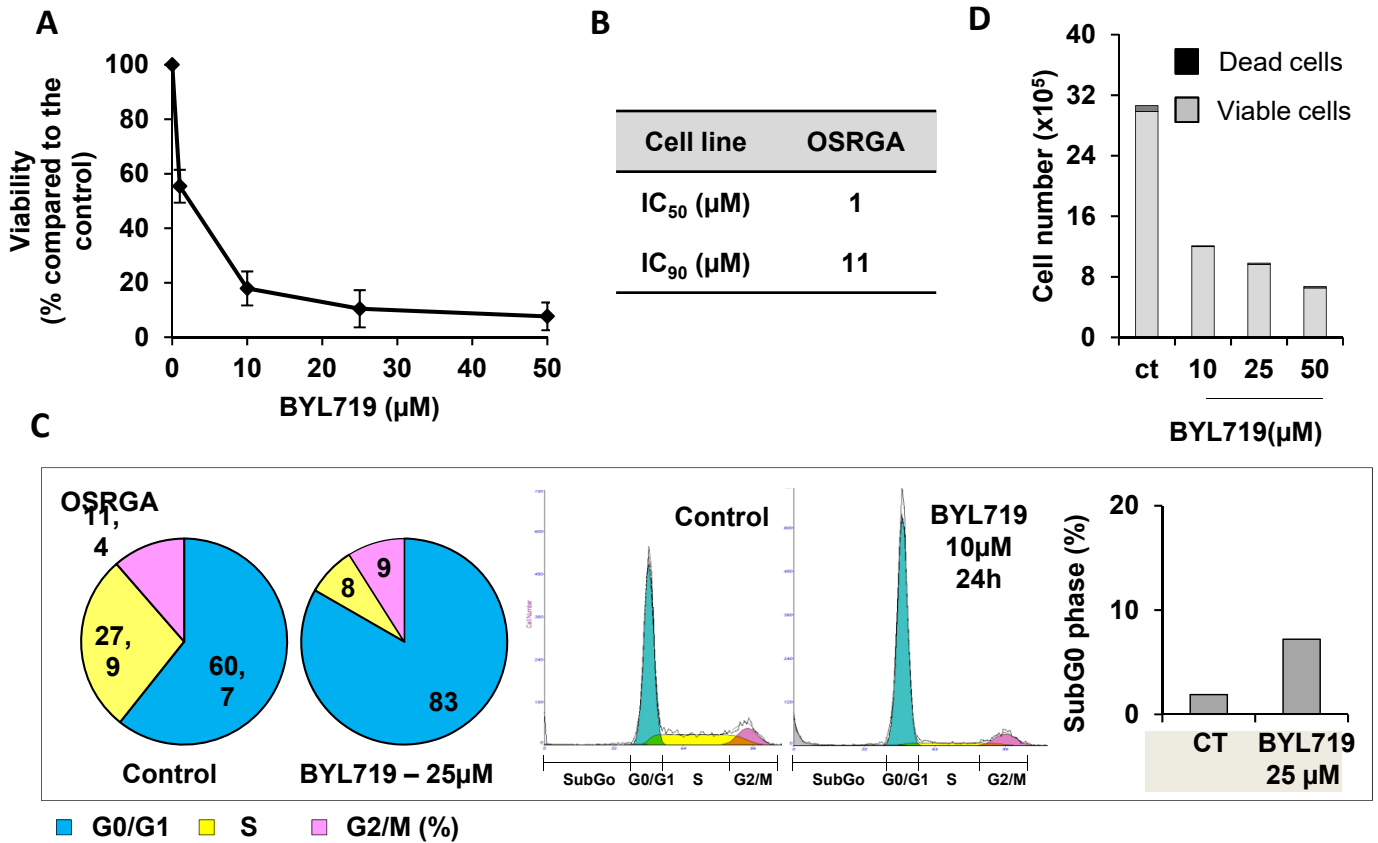
Features of gene specific forward (for) and reverse (rev) primers used for RT-PCR analysis. Primers were designed with Primer 0.5 software (Whitehead Institute for Biomedical Research). Reaction products were characterized by melting point determination (55-95°C with 0.5°C sec<sup>-1</sup>).

Supplementary data 3



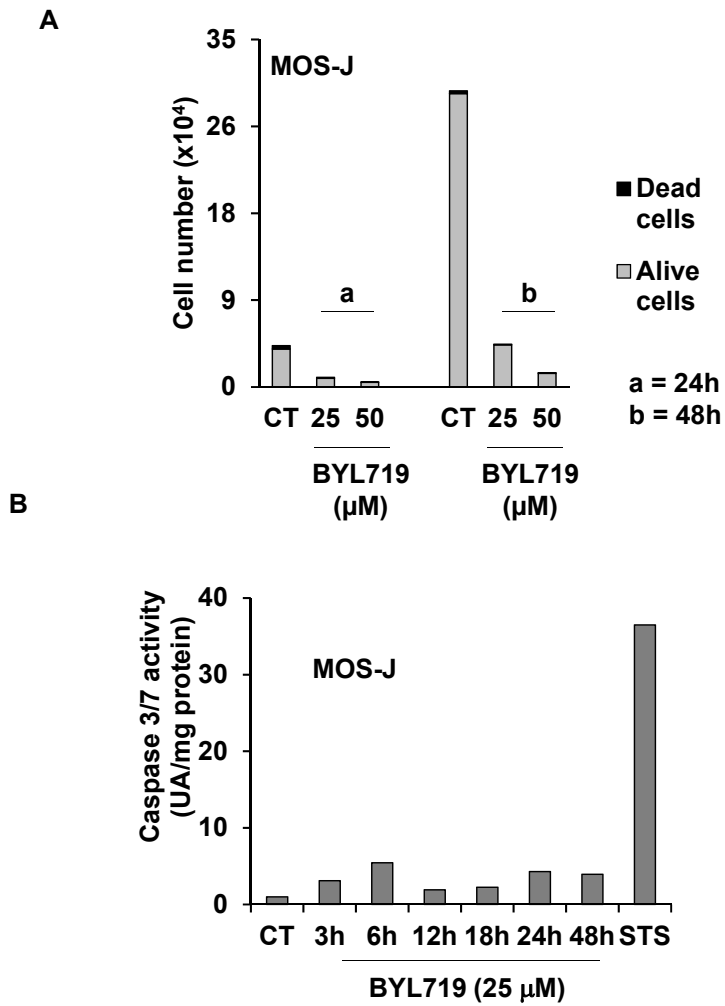
**Supplementary data 3 : BYL719 induces a cell cycle arrest in the G0/G1 phase of human and murine osteosarcoma cells.** Human (A) and murine (B) osteosarcoma cells were treated with 25 µM of BYL719 or vehicle (CT) for 18 hours before DNA labeling using propidium iodide and cell cycle analysis using flow cytometry. All experiments were repeated 3 times, and representative results are shown.

Supplementary Data 4



**Supplementary data 4: Effect of BYL719 on rat osteosarcoma cell line (OSRGA).** BYL719 decreased OSRGA cell viability after 72 hours of treatment (A). Data are presented as the percentage of untreated control cells and are expressed as the mean ± SD (standard deviation) of 3 independent experiments. (B) Summary of IC<sub>50</sub> and IC<sub>90</sub> for OSRGA cells. (C) BYL719 induced a blockade in G0/G1 phase of OSRGA cell line after 24h of treatment without inducing subG0 cells accumulation. (D) Manual counting of viable and dead OSRGA cells treated or not with BYL719 confirmed that BYL719 did not induce osteosarcoma cell death.

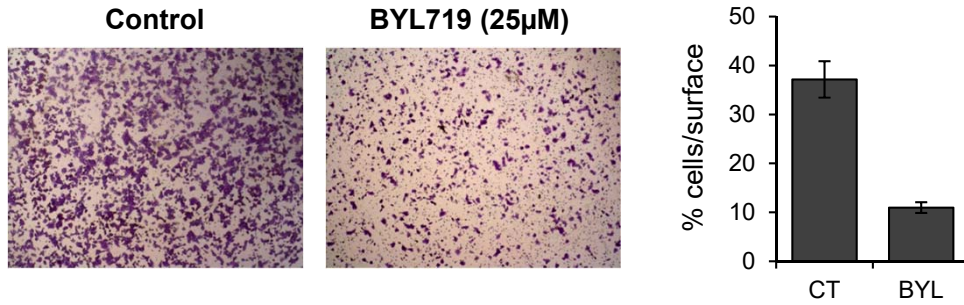
Supplementary Data 5



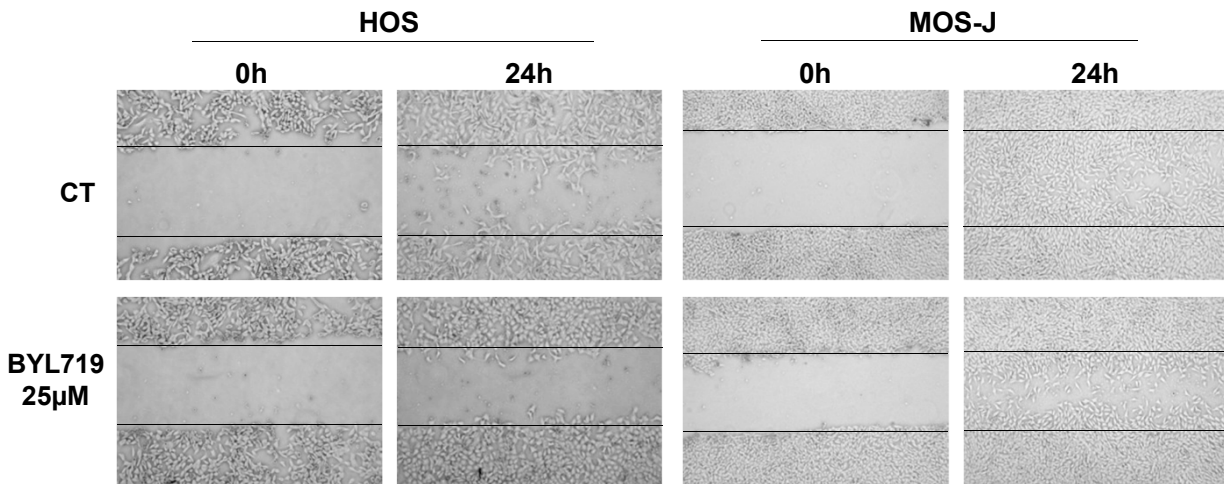
**Supplementary data 5: BYL179 does not induce osteosarcoma cell death. (A)** Manual cell counting of both alive (grey) and dead cells (black) after 24 and 48 hours of treatment with BYL719. **(B)** Caspase activity assay in a time course experiment with 25  $\mu\text{M}$  of BYL719, STS (Staurosporin) was used as positive control of caspase activity.

## Supplementary Data 6

**A**



**B**



### Supplementary data 6: BYL719 inhibits cell migration of osteosarcoma cell lines.

A, HOS-MMNG tumor cells were pretreated with +/- 25µM BYL719 for 6 hours, and then 150,000 tumor cells were seeded in boyden chambers with FBS gradient for 6 hours. After crystal violet staining, the migration was estimated in % of cells per surface using ImageJ software. B, the cells were cultured in 6-well plates until confluent before incubation with 4 µg/ml of mitomycin C (Sigma) for 1 hour to block cell proliferation. A slit was then made in the cell monolayer and cells were treated or not with BYL719 (25 µmol/L) for 24 h and phase-contrast photographs were taken at 0 and 24 hours to monitor cell migration. The horizontal bars represent the limit of the slit performed on the cell monolayer at the start of the experiment. Original magnification X 100.

## Supplementary Data 7

### Trabecular Bone

Parameters	Control	BYL719 (50 mg/kg/day)
TV	0.74 ± 0.08	0.82 ± 0.10
BV	0.20 ± 0.02	0.22 ± 0.02
BV/TV	27.53 ± 1.11	27.11 ± 1.23
TS	6.33 ± 0.35	7.10 ± 0.57
BS	10.55 ± 0.90	11.83 ± 1.19
BS/BV	52.89 ± 0.89	54.39 ± 1.42
BS/TV	14.52 ± 0.47	14.64 ± 0.41
Tb.Th	0.07 ± 0.002	0.07 ± 0.002
Tb.N	3.85 ± 0.12	3.82 ± 0.12
Tb.Sp	0.14 ± 0.004	0.15 ± 0.004

### Cortical Bone

Parameters	Control	BYL719 (50 mg/kg/day)
TV	7.33 ± 0.18	6.59 ± 0.13**
BV	5.04 ± 0.17	4.36 ± 0.12**
BV/TV	68.69 ± 0.57	66.02 ± 0.59**
TS	55.72 ± 0.59	55.28 ± 0.48
BS	57.85 ± 1.07	57.39 ± 0.68
BS/BV	11.52 ± 0.20	13.22 ± 0.33***
BS/TV	7.91 ± 0.08	8.72 ± 0.14***
CTh	0.24 ± 0.01	0.21 ± 0.004**

**Supplementary data 7: Histomorphometric parameters of contralateral trabecular and cortical bone in MOS-J osteoblastic osteosarcoma model (C57Bl/6J mice).** TV (tissue volume, mm<sup>3</sup>). BV (bone volume, mm<sup>3</sup>). BV/TV (percent of bone volume, %). TS (tissue surface, mm<sup>2</sup>). BS (bone surface, mm<sup>2</sup>). BS/BV (bone surface/bone volume ratio, mm<sup>-1</sup>). BS/TV (bone surface density, mm<sup>-1</sup>). CTh (cortical thickness, mm). TbTh (trabecular thickness, mm). TbN (trabecular number). TbSp (trabecular spaces, mm). \*P<0.05; \*\*P<0.01. \*\*\*P<0.001 compared to the control group.

## Supplementary Data 8

### Trabecular Bone

Parameters	Control	BYL719 (50 mg/Kg/day)
TV	0.59 ± 0.07	0.79 ± 0.06*
BV	0.15 ± 0.02	0.19 ± 0.01
BV/TV	26.15 ± 0.87	24.04 ± 1.51
TS	5.29 ± 0.46	7.37 ± 0.43 **
BS	6.84 ± 0.69	10.13 ± 0.82**
BS/BV	45.87 ± 1.01	53.76 ± 1.27***
BS/TV	11.99 ± 0.47	12.81 ± 0.57
Tb.Th	0.08 ± 0.002	0.07 ± 0.001*
Tb.N	3.22 ± 0.12	3.36 ± 0.16
Tb.Sp	0.20 ± 0.01	0.18 ± 0.1

### Cortical Bone

Parameters	Control	BYL719 (50 mg/Kg/day)
TV	5.73 ± 0.26	5.21 ± 0.09
BV	3.96 ± 0.14	3.52 ± 0.06 *
BV/TV	69.46 ± 1.33	67.81 ± 1.41
TS	35.52 ± 1.02	35.43 ± 0.72
BS	34.49 ± 0.81	34.42 ± 0.82
BS/BV	8.74 ± 0.21	9.77 ± 0.16 **
BS/TV	6.07 ± 0.18	6.62 ± 0.16 *
CTh	0.32 ± 0.01	0.28 ± 0.01 **

**Supplementary data 8: Histomorphometric parameters of contralateral trabecular and cortical bone in HOS xenogenic and osteoblastic osteosarcoma model (Nude mice).** TV (tissue volume, mm<sup>3</sup>). BV (bone volume, mm<sup>3</sup>). BV/TV (percent of bone volume, %). TS (tissue surface, mm<sup>2</sup>). BS (bone surface, mm<sup>2</sup>). BS/BV (bone surface/bone volume ratio, mm<sup>-1</sup>). BS/TV (bone surface density, mm<sup>-1</sup>). CTh (cortical thickness, mm). TbTh (trabecular thickness, mm). TbN (trabecular number). TbSp (trabecular spaces, mm). \*P<0.05; \*\*P<0.01. \*\*\*P<0.001 compared to the control group.