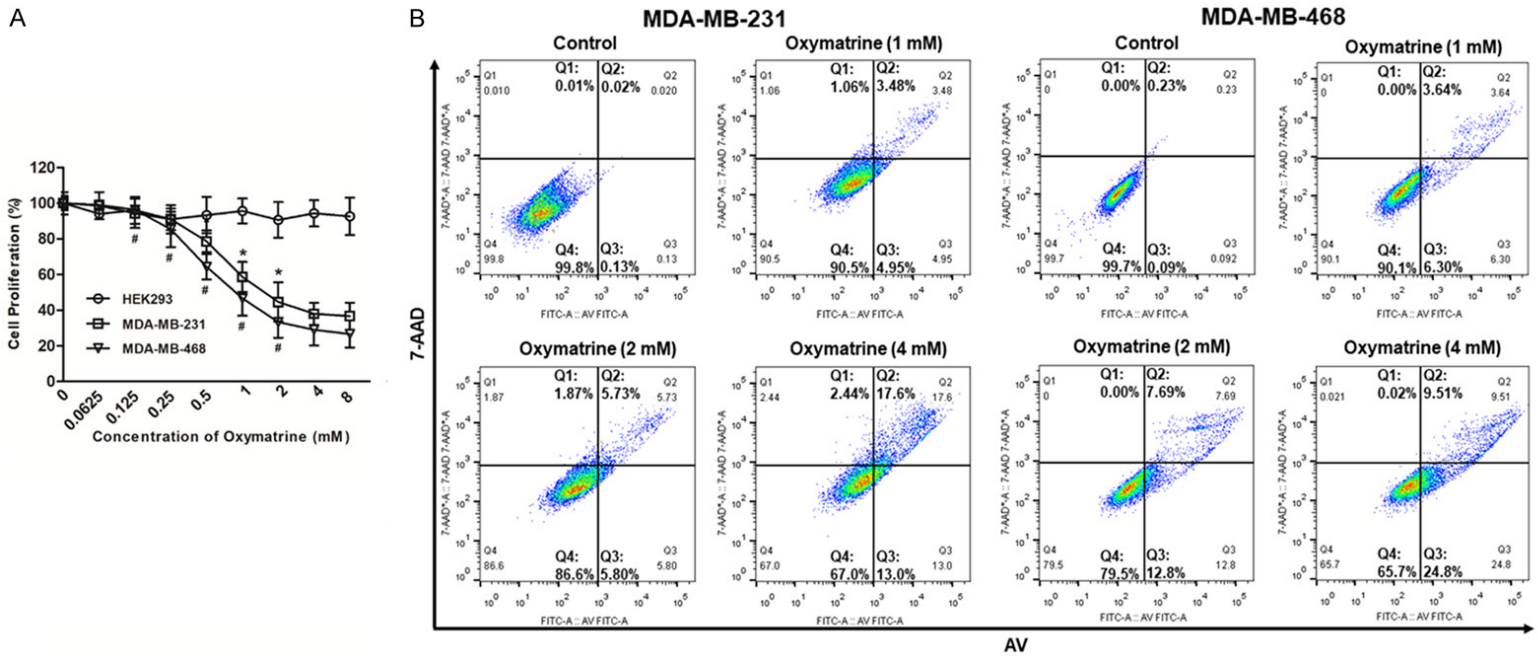
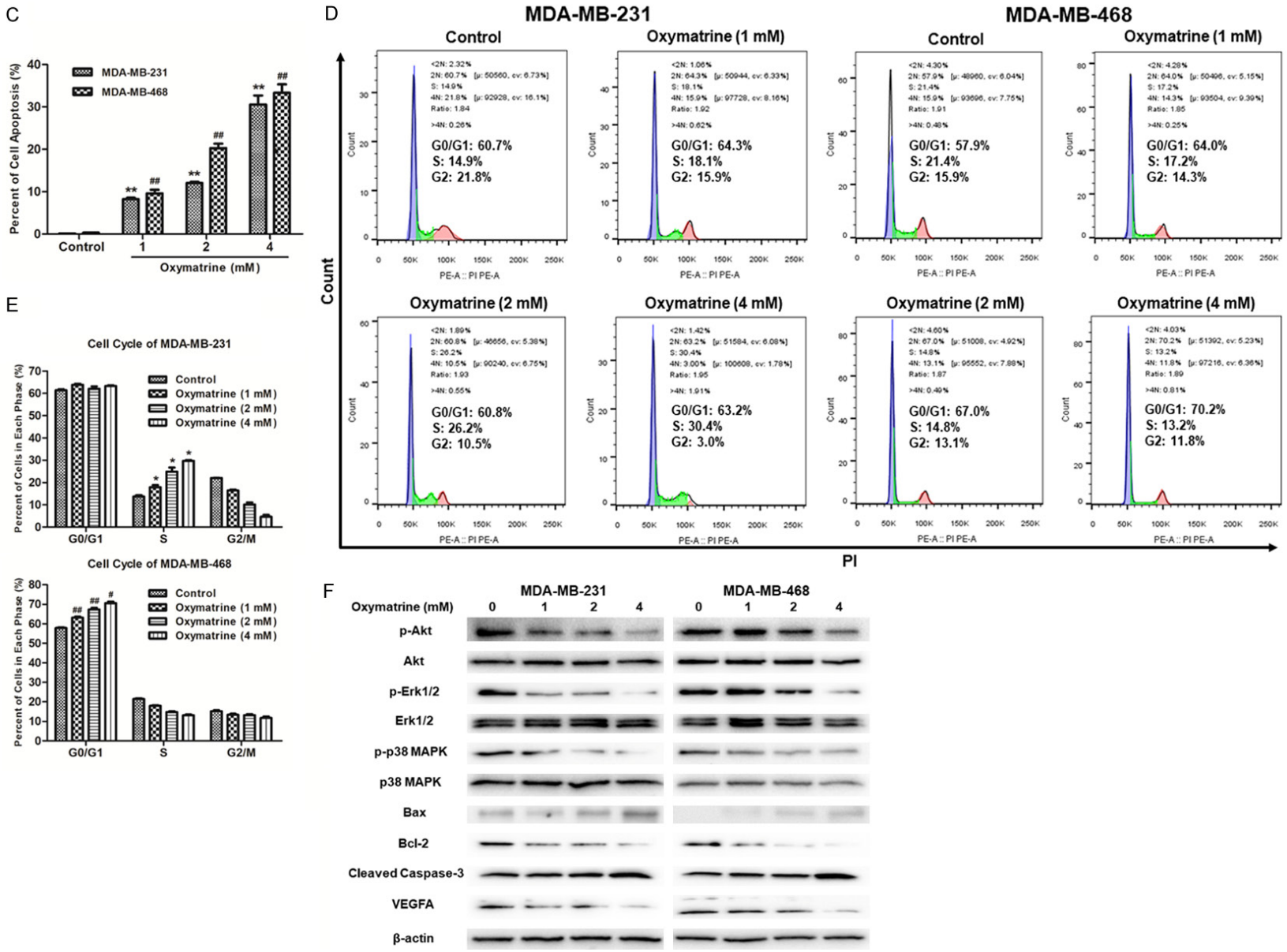

A
Cell Proliferation (%)
120
100
80
60
40
20
0
HEK293
MDA-MB-231
MDA-MB-468
0
0.0625
0.125
0.25
0.5
1
2
4
8
Concentration of Oxymatrine (mM)
B
MDA-MB-231
MDA-MB-468
Control
Oxymatrine (1 mM)
Oxymatrine (2 mM)
Oxymatrine (4 mM)
Q1: 0.01%
Q2: 0.02%
Q4: 99.8%
Q3: 0.13%
Q1: 1.06%
Q2: 3.48%
Q4: 90.5%
Q3: 4.95%
Q1: 1.87%
Q2: 5.73%
Q4: 86.6%
Q3: 5.80%
Q1: 2.44%
Q2: 17.6%
Q4: 67.0%
Q3: 13.0%
Q1: 0.00%
Q2: 0.23%
Q4: 99.7%
Q3: 0.09%
Q1: 0.00%
Q2: 3.64%
Q4: 90.1%
Q3: 6.30%
Q1: 0.00%
Q2: 7.69%
Q4: 79.5%
Q3: 12.8%
Q1: 0.02%
Q2: 9.51%
Q4: 65.7%
Q3: 24.8%
7-AAD
AV
FITC-A :: AV FITC-A
7-AAD*-A :: 7-AAD 7-AAD*-A


C
Percent of Cell Apoptosis (%)
MDA-MB-231
MDA-MB-468
Control
Oxymatrine (mM)
D
MDA-MB-231
MDA-MB-468
Control
Oxymatrine (1 mM)
Oxymatrine (2 mM)
Oxymatrine (4 mM)
G0/G1: 60.7%
S: 14.9%
G2: 21.8%
G0/G1: 64.3%
S: 18.1%
G2: 15.9%
G0/G1: 57.9%
S: 21.4%
G2: 15.9%
G0/G1: 64.0%
S: 17.2%
G2: 14.3%
G0/G1: 60.8%
S: 26.2%
G2: 10.5%
G0/G1: 63.2%
S: 30.4%
G2: 3.0%
G0/G1: 67.0%
S: 14.8%
G2: 13.1%
G0/G1: 70.2%
S: 13.2%
G2: 11.8%
Count
PI
PE-A :: PI PE-A
E
Cell Cycle of MDA-MB-231
Cell Cycle of MDA-MB-468
Percent of Cells in Each Phase (%)
G0/G1
S
G2/M
F
MDA-MB-231
MDA-MB-468
Oxymatrine (mM)
p-Akt
Akt
p-Erk1/2
Erk1/2
p-p38 MAPK
p38 MAPK
Bax
Bcl-2
Cleaved Caspase-3
VEGFA
β-actin

Supplementary Figure 1. Oxymatrine inhibited the proliferation and induced the apoptosis of TNBC cells. A. The viability of MDA-MB-231/MDA-MB-468 cells was assessed by MTT assay at 48 h after treatment with different concentrations of Oxymatrine. HEK293 cell was set as a negative control. Oxymatrine effectively inhibited the growth of MDA-MB-231/MDA-MB-468 cells in a dose-dependent manner, and the IC_{50} value was 0.57 mM/0.68 mM respectively. B. Representative plots showing the apoptosis patterns of Oxymatrine treated MDA-MB-231/MDA-MB-468 cells, the percentage of cells in each quadrant was indicated. C. Quantitative analysis of apoptosis assay. D. Representative plots of the cell cycle phases following different concentrations of Oxymatrine treated MDA-MB-231/MDA-MB-468 cells, the percentage of cells in each phase was indicated. E. Statistical analysis of cell numbers at different cell cycle phases. Data were presented as the mean ± SD, n = 3, $^{*}P < 0.05$, $^{**}P < 0.01$ (MDA-MB-231 cells), *vs*. the previous group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ (MDA-MB-468 cells), *vs*. the previous group. Oxymatrine promoted S-phase arrest in MDA-MB-231 cells. Unlike MDA-MB-231 cells, Oxymatrine promoted G0/G1-phase arrest in MDA-MB-468 cells. The Oxymatrine-induced S-phase or G0/G1-phase arrest probably accounted for the suppression of cell proliferation and induction of cell apoptosis. F. Preliminary study on the anti-tumor mechanism of Oxymatrine *via* Western blot. MDA-MB-231/MDA-MB-468 cells were incubated with different concentrations of oxymatrine (0, 1, 2 and 4 mg/mL) for 48 h. The protein expression levels of p-Akt/Akt, p-Erk/Erk, p-p38/p38, VEGFA and the apoptosis-related protein expression levels of Cleaved Caspase-3, Bax, Bcl-2 were evaluated by Western blot. Equal loading of protein was confirmed by stripping the immunoblot and reprobing it for β-actin. Oxymatrine significantly interfered with the phosphorylation of Akt, Erk and p38 MAPK in a dose-dependent manner, strongly suggesting that Oxymatrine inhibited proliferation and migration of TNBC cells by suppressing the activities of Akt, Erk and p38 MAPK protein kinase. Subsquently, examination of apoptosis-associated proteins showed that Oxymatrine treatment greatly reduced the expression of anti-apoptotic protein Bcl-2, also increased the expression of pro-apoptotic protein Bax. In addition, the immunoblot of cleaved Caspase-3 further verified that, Oxymatrine could induce the apoptosis of TNBC cells. It was noteworthy that, consistent with the anti-angiogenetic potency *in vitro*, Oxymatrine also significantly decreased the level of cytoplasmatic pro-angiogenic VEGF, which needed to be further confirmed in tumor tissue samples.