SUPPLEMENTARY MATERIAL

Figure S1. Structure and cytotoxicity of axitinib alone in drug-resistant cancer cells and their parental drug sensitive cells, SP cells and non-SP cells.

Figure S2. The expression of ABCG2, ABCB1 and ABCC1 in drug resistant and their parental sensitive cell lines. The protein expression of ABCG2, ABCB1 and ABCC1 in S1 (lane 1), HEK293/pcDNA3.1 (lane 2), ABCG2-482-G2 (lane 3) , ABCG2-482-T7 (lane 4), S1-M1-80 (lane 5), KB (lane 6) , KBv200 (lane 7), HL60 (lane 8) and HL60/ADR (lane 9) cell lines without treatment were measured by western blot analysis.

Figure S3. Potentiation of the antitumor effects of topotecan by axitinib in S1 cell xenograft model in athymic nude mice. (A) Changes in tumor volume with time. Each point represents the mean ± SD of tumor volumes from ten mice in the group. (B) Tumor size. The picture was taken on the 22th day after implantation. (C) Changes in body weight with time after tumor cell inoculation. Each point represents the mean ± SD of body weight from ten mice in the group. The various treatments were as follows: (a) saline (q4d × 4, i.p.); (b) topotecan (q4d × 4, i.p., 3 mg/kg); (c) axitinib (q4d × 4, p.o., 25 mg/kg); (d) topotecan (q4d × 4, i.p., 3 mg/kg) and axitinib (q4d × 4, p.o., 25 mg/kg) (axitinib was given one hour before topotecan administration).

Figure S4. SP cells were more tumorigenic. (A) Tumorigenicity was examined using (NOD/SCID) mice, into which 1×10^3-1×10^6 SP or non-SP cells of A549 origin were subcutaneously transplanted. (B) Tumor size. The picture was taken on the 44th day after

implantation.

Figure S5. Axitinib targeted to SP cells and enhanced the effect of chemotherapeutical agents on the induction of apoptosis.

Table 1. Effect of axitinib on reversing ABCG2-mediated drug resistance

Compounds	IC_{50} ± SD (μmol/L) (fold-reversal)			
	S1		S1-M1-80(ABCG2)	
Topetecan	0.250 ± 0.014	(1.00)	12.79 ± 0.241	(1.00)
+ 0.25 μM Axitinib	0.249 ± 0.020	(1.01)	6.692 ± 0.504**	(1.85)
+ 0.5 μM Axitinib	0.255 ± 0.017	(0.98)	4.964 ± 0.320**	(2.47)
+ 1.0 μM Axitinib	0.199 ± 0.006	(1.26)	3.020 ± 0.242**	(4.11)
+ 0.5 μM FTC	0.244 ± 0.013	(1.02)	1.195 ± 0.108**	(10.7)
Mitoxantrone	0.193 ± 0.021	(1.00)	14.668 ± 0.636	(1.00)
+ 0.25 μM Axitinib	0.194 ± 0.057	(0.99)	7.992 ± 0.675**	(1.84)
+ 0.5 μM Axitinib	0.201 ± 0.011	(0.96)	4.140 ± 0.301**	(3.54)
+ 1.0 μM Axitinib	0.185 ± 0.011	(1.12)	2.904 ±0.482**	(5.05)
+ 0.5 μM FTC	0.188 ± 0.011	(1.03)	1.272 ± 0.056**	(11.5)
Cisplatin	14.877 ± 1.126	(1.00)	14.496 ± 1.112	(1.00)
+ 1.0 μM Axitinib	14.206 ± 1.460	(1.05)	14.360 ± 1.812	(1.01)
	KB		KBv200(ABCB1)	
Doxorubicin	0.024 ± 0.002	(1.00)	2.204 ± 0.108	(1.00)
+ 0.25 μM Axitinib	0.024 ± 0.002	(1.00)	2.022 ± 0.185	(1.08)
+ 0.5 μM Axitinib	0.025 ± 0.001	(0.96)	2.153 ± 0.146	(1.02)
+ 1.0 μM Axitinib	0.015 ± 0.004*	(1.63)	1.250 ± 0.215*	(1.76)
+ 10 μM Verapamil	0.024 ± 0.003	(1.00)	0.109 ± 0.008**	(20.2)
Cispatin	1.552 ± 0.121	(1.00)	2.364 ± 0.241	(1.00)
+ 1.0 μM Axitinib	1.514 ± 0.117	(1.03)	2.412 ± 0.225	(0.98)
	HL60		HL60/ADR(ABCC1)	
Doxorubicin	0.055 ± 0.011	(1.00)	5.725 ± 0.203	(1.00)
+ 0.1 μM Axitinib	0.054 ± 0.006	(1.02)	5.719 ± 0.591	(1.00)
+ 0.2 μM Axitinib	0.056 ± 0.006	(0.98)	5.759 ± 0.659	(0.99)
+ 0.4 μM Axitinib	0.049 ± 0.005	(1.13)	5.476 ± 0.384	(1.04)
+ 40 μM MK571	0.054 ± 0.005	(1.02)	1.108 ± 0.182**	(5.17)

Cisplatin	1.456 ± 0.187	(1.00)	1.658 ± 0.118	(1.00)
+ 0.4 μM Axitinib	1.516 ± 0.125	(0.96)	1.524 ± 0.175	(1.08)
	SW1573		SW1573/2R120(LRP)	
Doxorubicin	0.108 ± 0.012	(1.00)	1.266 ± 0.121	(1.00)
+ 0.25 μM Axitinib	0.096 ± 0.004	(1.12)	1.214 ± 0.174	(1.04)
+ 0.5 μM Axitinib	0.098 ± 0.004	(1.10)	1.236 ± 0.122	(1.02)
+ 1.0 μM Axitinib	0.094 ± 0.006	(1.14)	1.138 ± 0.088	(1.11)
Cisplatin	2.473 ± 0.167	(1.00)	9.659 ± 1.073	(1.00)
+ 1.0 μM Axitinib	2.790 ± 0.147	(0.89)	9.180 ± 0.881	(1.05)
	NIH3T3		NIH3T3/MRP4-2(ABCC4)	
6-MP	0.298 ± 0.050	(1.00)	11.852 ± 1.564	(1.00)
+ 0.25 μM Axitinib	0.285 ± 0.034	(1.04)	11.145 ± 1.048	(1.06)
+ 0.5 μM Axitinib	0.318 ± 0.045	(0.94)	10.709 ± 1.637	(1.11)
+ 1.0 μM Axitinib	0.265 ± 0.011	(1.12)	12.319 ± 1.704	(0.96)
Cisplatin	6.140 ± 0.728	(1.00)	5.668 ± 0.690	(1.00)
+ 1.0 μM Axitinib	5.033 ± 0.296	(1.21)	5.842 ± 0.691	(0.97)

Cell survival was determined by MTT assays as described in "Materials and Methods". Data are the mean ± standard deviation (SD) of at least three independent experiments performed in triplicate. The fold-reversal of MDR (values given in parenthesis in last column) was calculated by dividing the IC_{50} for cells with the anticancer drugs in the absence of axitinib by that obtained in the presence of axitinib.

*$P < 0.05$, **$P < 0.01$ versus the values obtained in the absence of inhibitor

Table 2. Effect of axitinib on reversing ABCG2-mediated MDR in transfected cell lines

Compounds	IC_{50} ± SD (μmol/L) (fold-reversal)					
	HEK293/pcDNA3.1		ABCG2-G482-G2		ABCG2-482-T7	
Mitoxantrone	0.029±0.0015	(1.00)	1.176±0.241	(1.00)	1.195±0.301	(1.00)
+ 0.25 μM Axitinib	0.031±0.0024	(0.94)	0.488±0.085**	(2.41)	0.338±0.028**	(3.54)
+ 0.5 μM Axitinib	0.041±0.0029	(0.71)	0.247±0.076**	(4.76)	0.206±0.013**	(5.80)
+ 1.0 μM Axitinib	0.042±0.0033	(0.70)	0.079±0.007**	(14.9)	0.076±0.005**	(15.7)
+ 0.5 μM FTC	0.040±0.0011	(0.73)	0.138±0.005**	(8.52)	0.096±0.003**	(12.4)
Cisplatin	4.069±0.258	(1.00)	5.141±0.197	(1.00)	4.324±0.176**	(1.00)
+ 1.0 μM Axitinib	5.449±0.269	(0.75)	4.512±0.224	(1.14)	5.078±0.209**	(0.85)

Cell survival was determined by MTT assays as described in "Materials and Methods". Data are the mean ± standard deviation (SD) of at least three independent experiments performed in triplicate. The fold-reversal of MDR (values given in parenthesis in last column) was calculated by dividing the IC_{50} for cells with the anticancer drugs in the absence of axitinib by that obtained in the presence of axitinib.

*$P < 0.05$, **$P < 0.01$ versus the values obtained in the absence of inhibitor

Figure S1

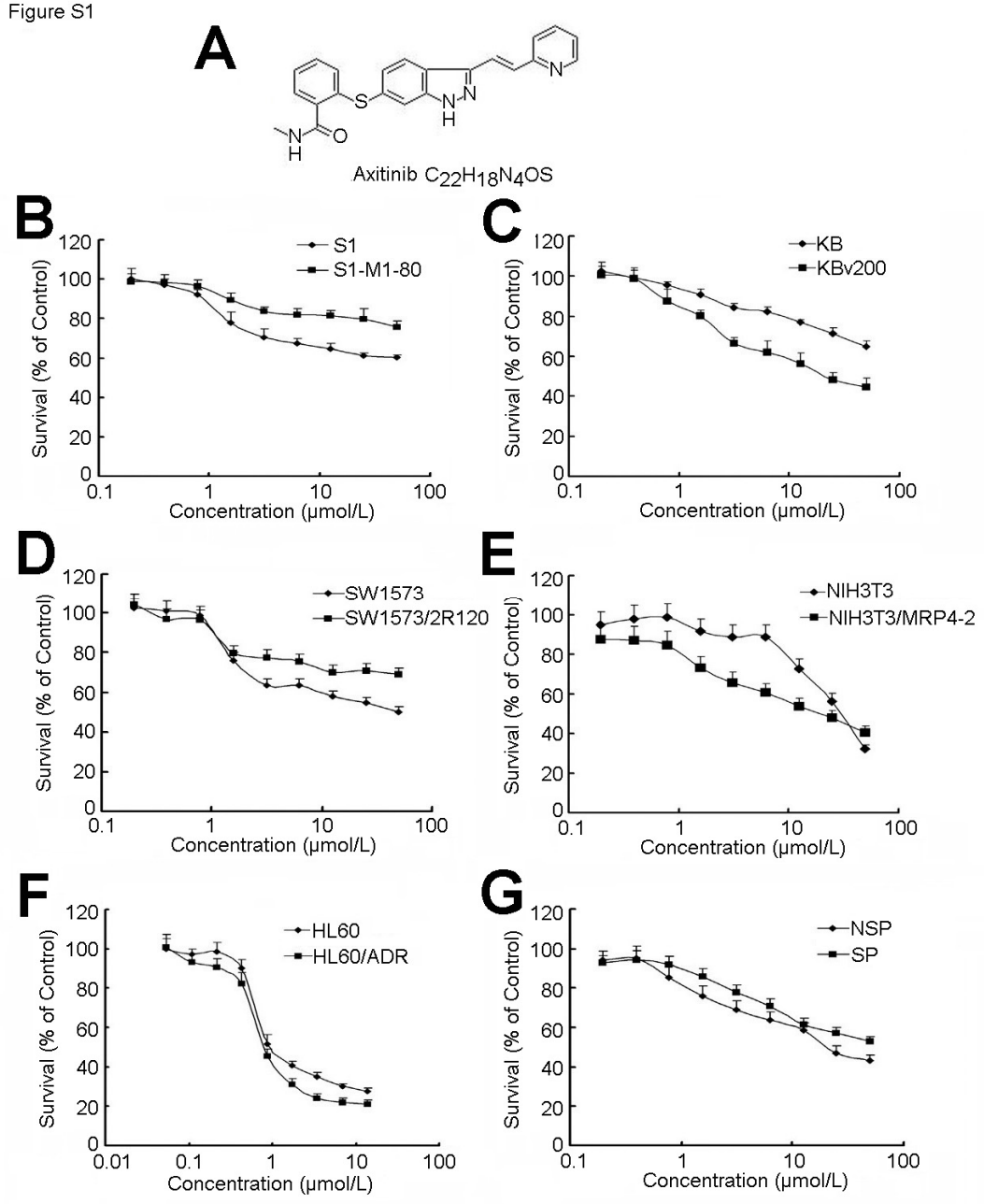

A
Axitinib $C_{22}H_{18}N_4OS$
B
S1
S1-M1-80
C
KB
KBv200
D
SW1573
SW1573/2R120
E
NIH3T3
NIH3T3/MRP4-2
F
HL60
HL60/ADR
G
NSP
SP
Survival (% of Control)
Concentration (μmol/L)

Figure S2

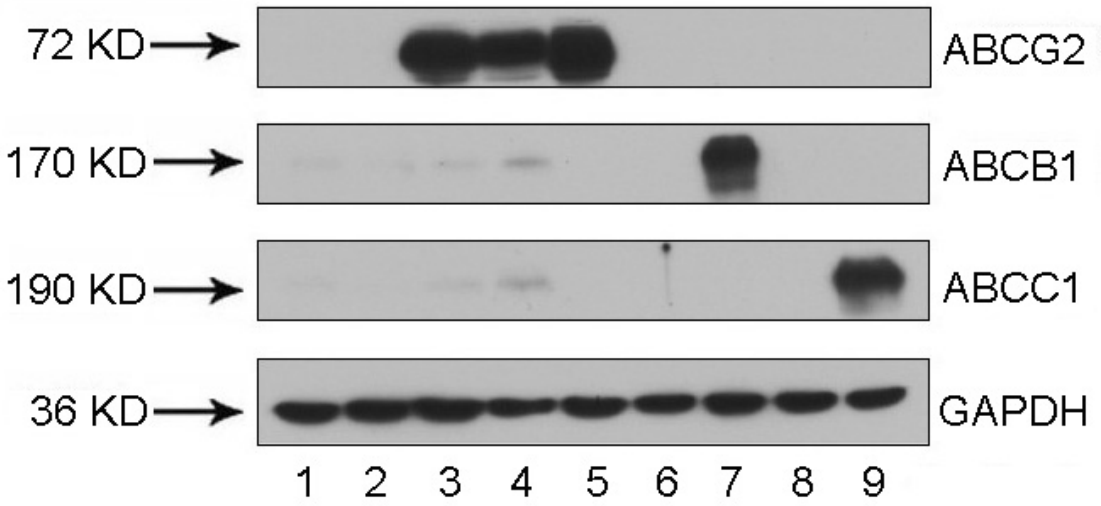


Figure S3

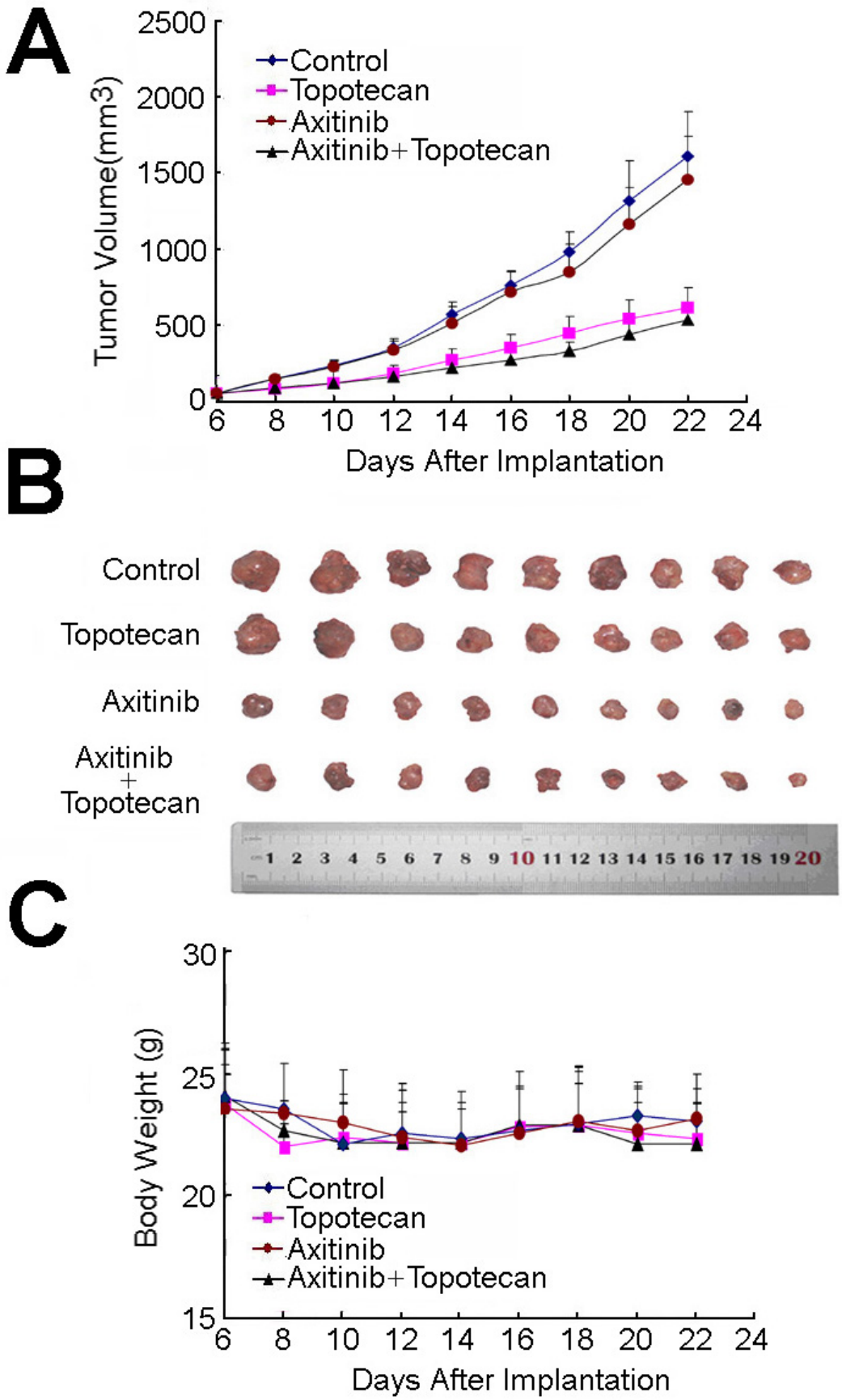

A
Tumor Volume(mm3)
2500
2000
1500
1000
500
0
6
8
10
12
14
16
18
20
22
24
Days After Implantation
Control
Topotecan
Axitinib
Axitinib+Topotecan
B
Control
Topotecan
Axitinib
Axitinib
+
Topotecan
C
Body Weight (g)
30
25
20
15
Days After Implantation
Control
Topotecan
Axitinib
Axitinib+Topotecan

Figure S4

A

Table 1. SP Is Enriched With Tumorigenic Cells

Cells	Cell number for injection (tumor volume mm^3)			
	1×10^6	1×10^5	1×10^4	1×10^3
SP	6/6 (389)	3/6 (59)	1/6 (39)	0/6
NSP	6/6 (107)	0/6	0/6	0/6

B

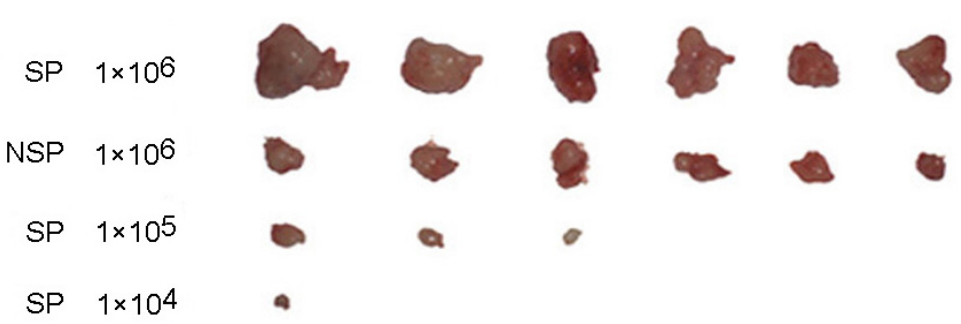
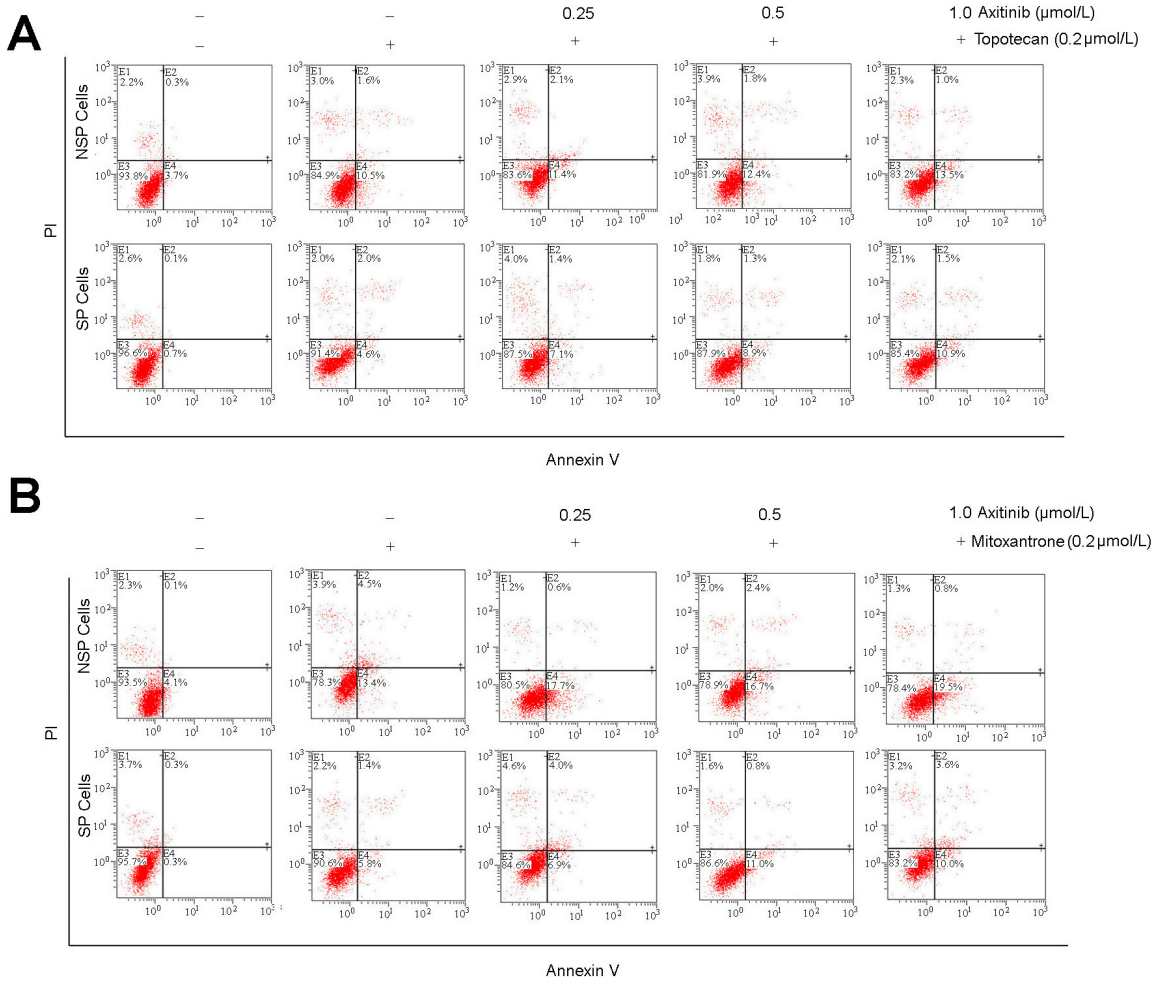


Figure S5


A
–
–
–
+
0.25
+
0.5
+
1.0 Axitinib (μmol/L)
+ Topotecan (0.2 μmol/L)
NSP Cells
SP Cells
PI
Annexin V
B
–
–
–
+
0.25
+
0.5
+
1.0 Axitinib (μmol/L)
+ Mitoxantrone (0.2 μmol/L)
NSP Cells
SP Cells
PI
Annexin V