Supplemental Figure Legend

Fig. S1. p53-independent PUMA induction by staurosporine in colon cancer cells. A. *p53*-KO HCT116 cells were treated with 60 nM staurosporine (STS). After total RNA was isolated at indicated time points, real-time RT PCR was used to analyze PUMA mRNA expression. **B.** PUMA protein expression at indicated time points in *p53*-KO HCT116 cells treated with 60 nM STS was probed by Western blotting. **C.** WT HCT116, *p53*-KO HCT116, and p53-deficient DLD1 cells were treated with increasing doses of STS for 24 hr. Western blotting was performed to analyze PUMA expression.

Fig. S2. Induction of PUMA by FoxO3a and inhibitors of the PI3K pathway. **A.** *p53*-KO HCT116 cells were transfected with FoxO3a triple mutant (FoxO3aTM) or the control empty vector for 24 hr. PUMA and FoxO3a expression was analyzed by Western blotting**. B**. WT and *p53*-KO HCT116 cells were treated for 24 hr with 5 μM wortmannin, 1 μM UCN-01, or 50 μM LY294002. PUMA expression was analyzed by Western blotting.

Fig. S3. UCN-01-induced and PUMA-dependent apoptosis in colon cancer cells. **A.** WT and *PUMA*-KO DLD1 colon cancer cells were treated with 3 μM UCN-01 for 48 hr. Apoptosis was analyzed by nuclear staining with Hoechst 33258. **B.** *p21*-KO and *p21*/*PUMA*-KO HCT116 cells were treated with 2 μM UCN-01 for 36 hr. Cells were stained with annexin V/PI and analyzed by flow cytometry. The percentages of Annexin-positive cells are indicated in the right quadrants. **C.** After treatment as in **B**, mitochondrial membrane potential was analyzed by mitotracker staining followed by flow cytometry. **D.** Left: Parental and stable *FoxO3a*-knockdown (*FoxO3a*-KD) *p21*-KO HCT116 cells were treated with 1 μM UCN-01 and blotted for PUMA and FoxO3a

expression. Right: Parental and *FoxO3a*-KD *p21*-KO cells were treated with 3 μM UCN-01 for 48 hr. Apoptosis was determined by nuclear staining.

Fig. S4. PUMA mediates the chemosensitization effect of UCN-01.

A. *p21*-KO and *p21*-KO/*PUMA*-KO HCT116 cells were treated with 50 μM cisplatin for 48 hr. Apoptosis was analyzed by nuclear staining. **B.** Caspase 3 activation in cells analyzed in **A**. **C.** *p21*-KO and *p21*-KO/*PUMA*-KO HCT116 cells were treated with 0.5 μM UCN-01, 50 μM cisplatin, or their combination for 48 hr. Caspase 3 activation was analyzed by Western blotting.

Fig. S5. PUMA-dependent apoptosis in xenograft tumors treated with UCN-01.

p21-KO or *p21*-KO/*PUMA*-KO HCT116 cells were treated with 9 mg/kg UCN-01 or control sodium citrate buffer by i.p. injection for 5 consecutive days as described in Figure 6. Paraffin-embedded tumor sections were analyzed by immunostaining. **A.** TUNEL staining (400×). **B.** Active caspase 3 staining (400×).

Figure S1

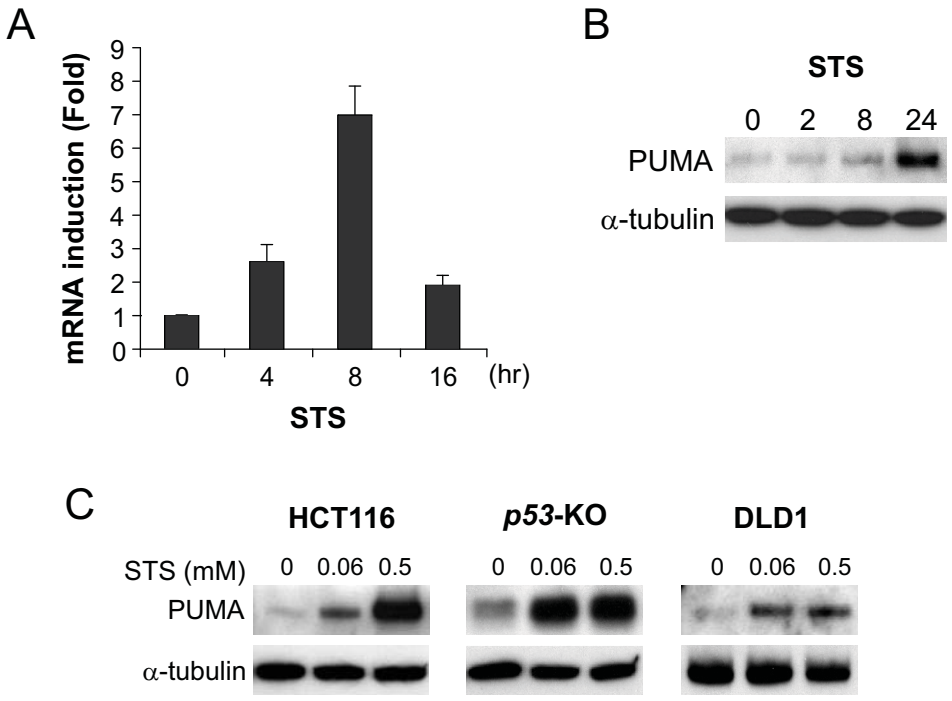

A
mRNA induction (Fold)
0
1
2
3
4
5
6
7
8
9
0
4
8
16
(hr)
STS
B
STS
0
2
8
24
PUMA
α-tubulin
C
HCT116
p53-KO
DLD1
STS (mM)
0
0.06
0.5
PUMA
α-tubulin

Figure S2

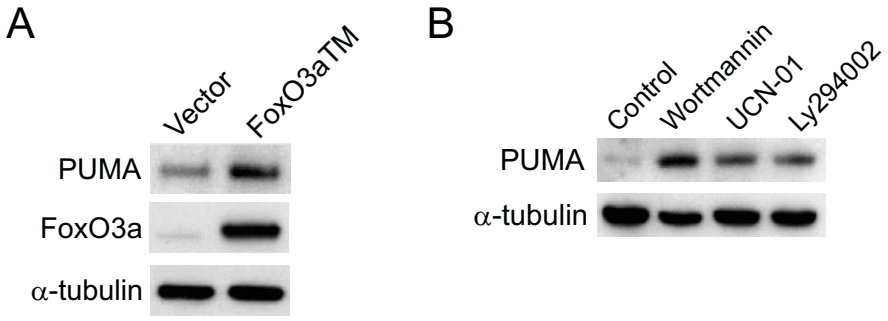

A
Vector
FoxO3aTM
PUMA
FoxO3a
α-tubulin
B
Control
Wortmannin
UCN-01
Ly294002
PUMA
α-tubulin

Figure S3

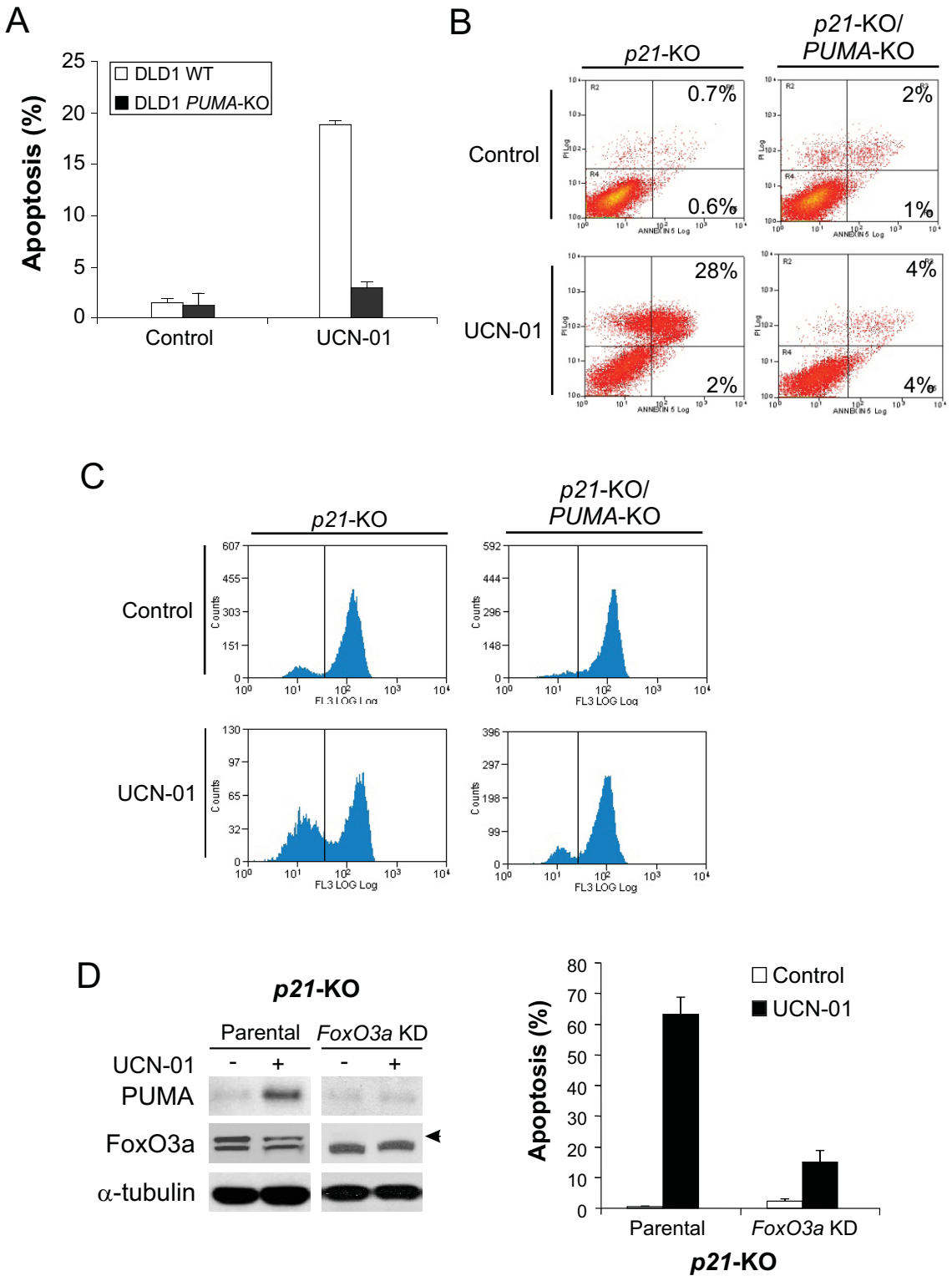

A
Apoptosis (%)
25
20
15
10
5
0
DLD1 WT
DLD1 PUMA-KO
Control
UCN-01
B
p21-KO
p21-KO/
PUMA-KO
Control
UCN-01
0.7%
0.6%
2%
1%
28%
2%
4%
4%
C
p21-KO
p21-KO/
PUMA-KO
Control
UCN-01
Counts
FL3 LOG Log
D
p21-KO
Parental
FoxO3a KD
UCN-01
-
+
-
+
PUMA
FoxO3a
α-tubulin
Apoptosis (%)
80
70
60
50
40
30
20
10
0
Control
UCN-01
Parental
FoxO3a KD
p21-KO

Figure S4

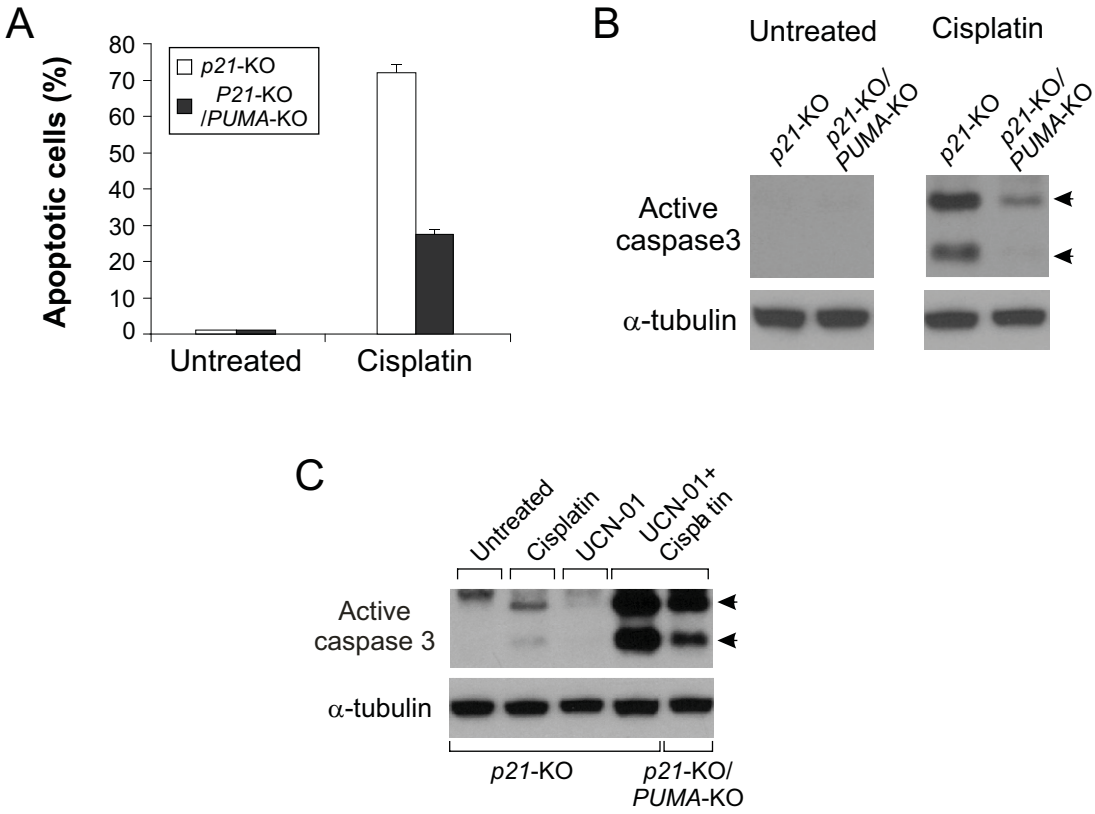
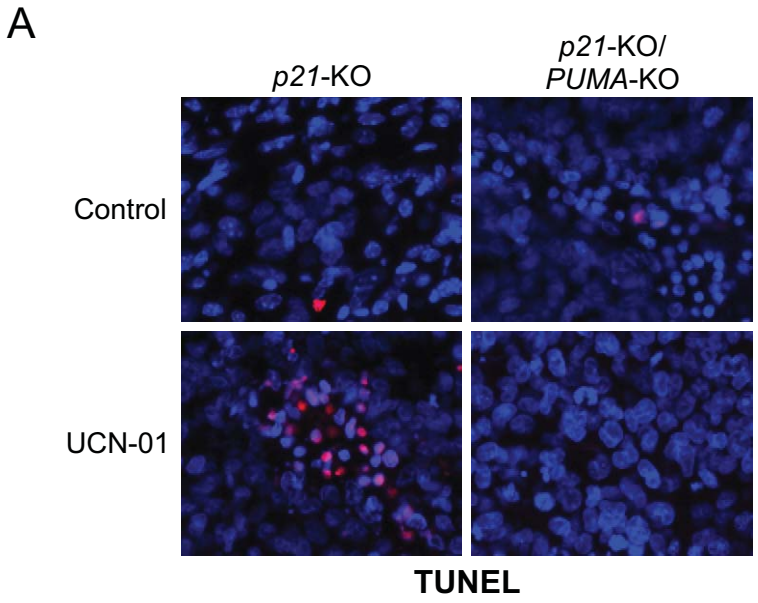
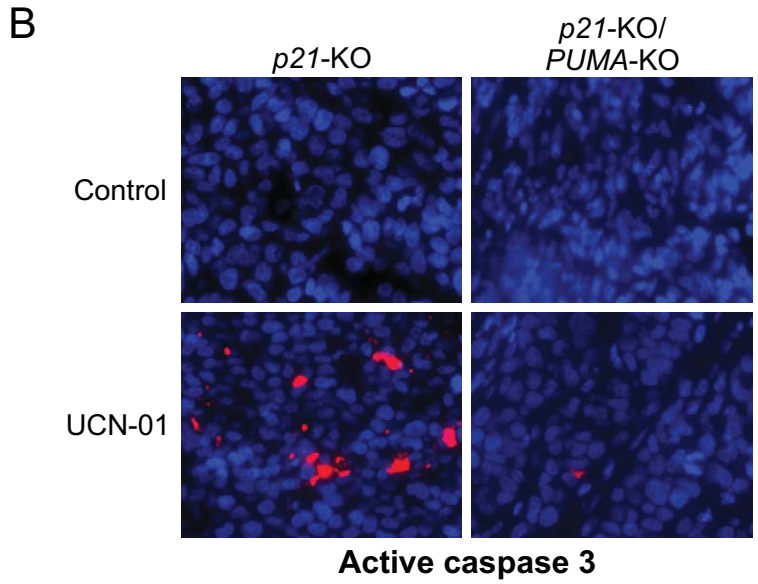

A
Apoptotic cells (%)
80
70
60
50
40
30
20
10
0
p21-KO
P21-KO /PUMA-KO
Untreated
Cisplatin
B
Untreated
Cisplatin
p21-KO
p21-KO/ PUMA-KO
p21-KO
p21-KO/ PUMA-KO
Active caspase3
α-tubulin
C
Untreated
Cisplatin
UCN-01
UCN-01+ Cispla tin
Active caspase 3
α-tubulin
p21-KO
p21-KO/ PUMA-KO

Figure S5


A
p21-KO
p21-KO/
PUMA-KO
Control
UCN-01
TUNEL


B
p21-KO
p21-KO/
PUMA-KO
Control
UCN-01
Active caspase 3