Table S1. Primary antibodies information

Target	Manufacturer	Species	Dilution factor
NOTCH1	Cell signaling #4380	Rabbit	1000
NICD1	Cell signaling #4147	Rabbit	1000
HES1	Cell signaling #11988	Rabbit	10000
HES5	Abcam #194111	Rabbit	10000
p53	BD Pharmingen #554293	Mouse	1000
p21	BD Pharmingen #556430	Mouse	1000
BAX	Cell signaling #5023	Rabbit	1000
NESTIN	Millipore #MAB5326	Mouse	1000
CD133	Abcam #16518	Rabbit	1000
PARP1	SCBT #7150	Rabbit	500
Cleaved PARP1	Cell signaling #5625	Rabbit	1000
Cleaved caspase-3	Cell signaling #9661	Rabbit	1000

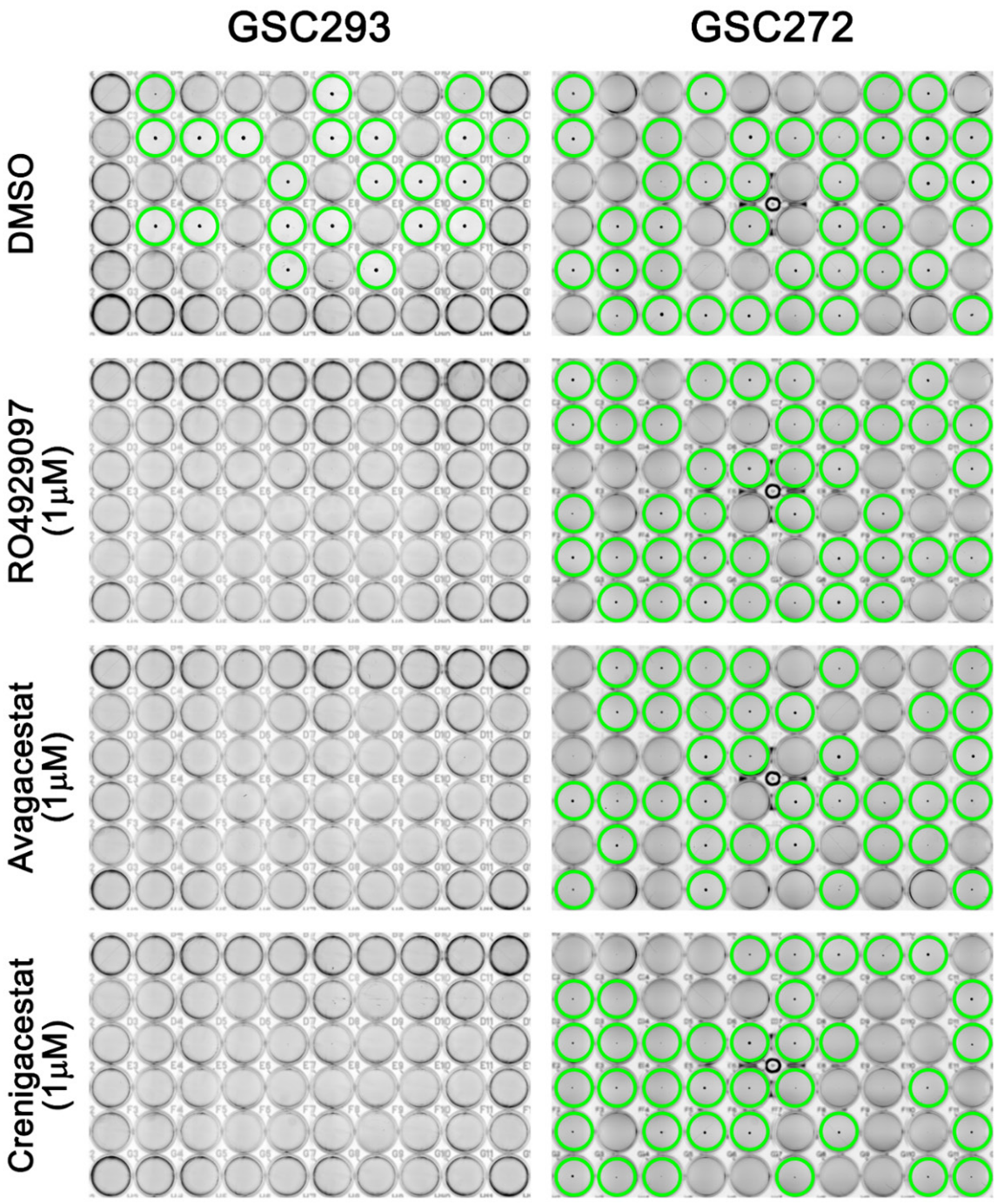


Figure S1. Representative images of GSC sphere formation after GSIs treatment. RO4929097, avagacestat, and crenigacestat treatment significantly attenuated sphere formation ability in GSC293 (wt-p53) but not in GSC272 (mut-p53). Wells with spheres were labeled by green circle. n = 3.

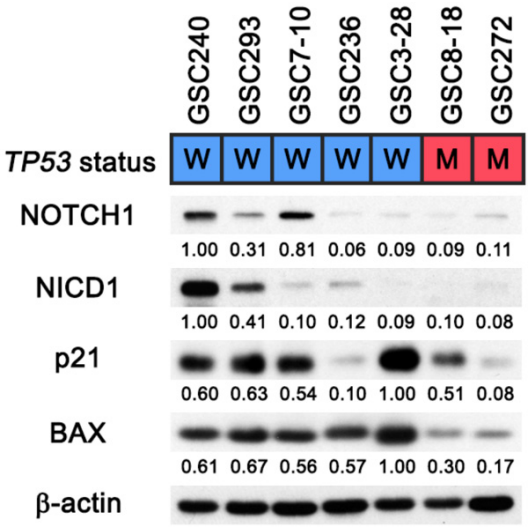


Figure S2. Expression of NOTCH1 signaling and p53 target genes in the validation group. The basal expression of NOTCH1 signaling (NOTCH1 and NICD1) and p53 target genes (p21 and BAX) was detected by Western blot analysis. *TP53* status (W: wild-type, M: mutant) is also shown. n = 3.

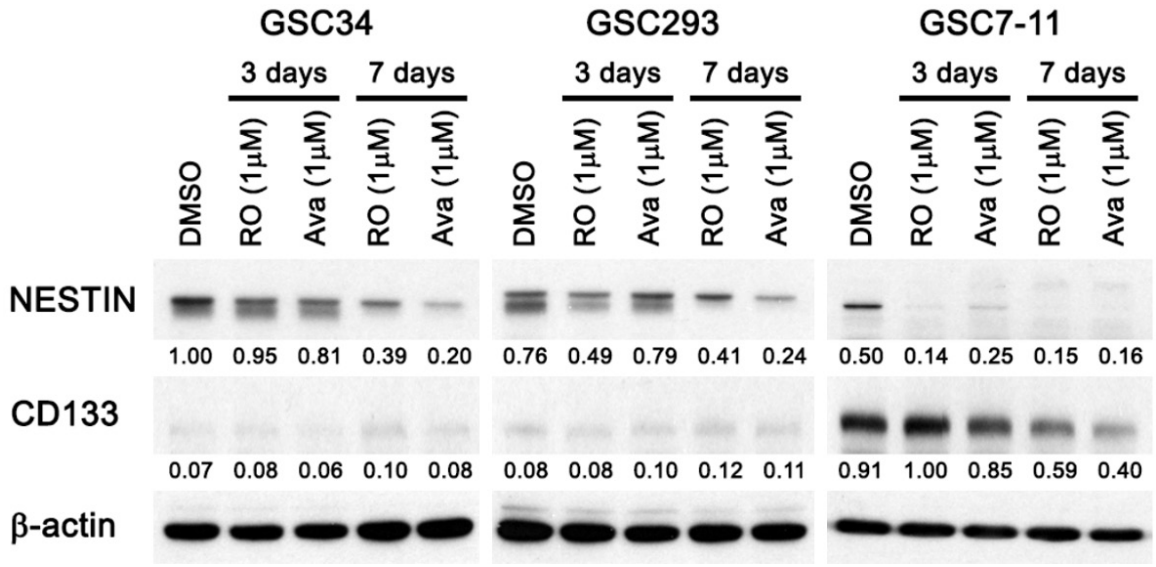


Figure S3. GSIs downregulated the expression of stem cell markers in GSCs. GSCs were treated by RO4929097 and avagacestat for 3 and 7 days. Both GSIs treatment decreased NESTIN and CD133 expression in GSCs. n = 3.

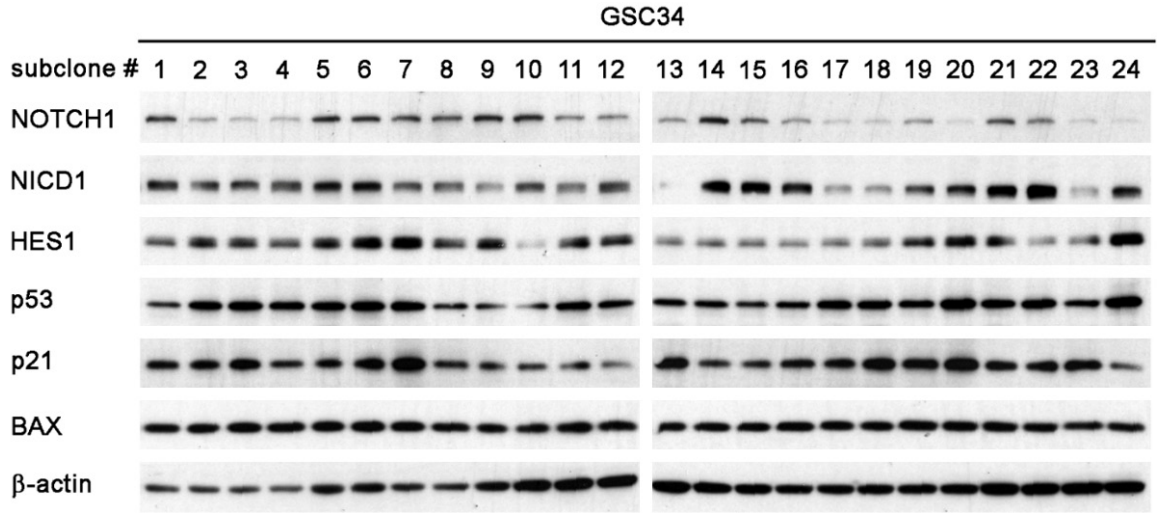


Figure S4. Expression of NOTCH1 and wt-p53 signaling in single cell-derived subclones of GSC34. Single cells from GSC34 were obtained by flowcytometry sorting. The single cell-derived subclones showed heterogenous expression pattern of NOTCH1 signaling and comparable wt-p53 activity.

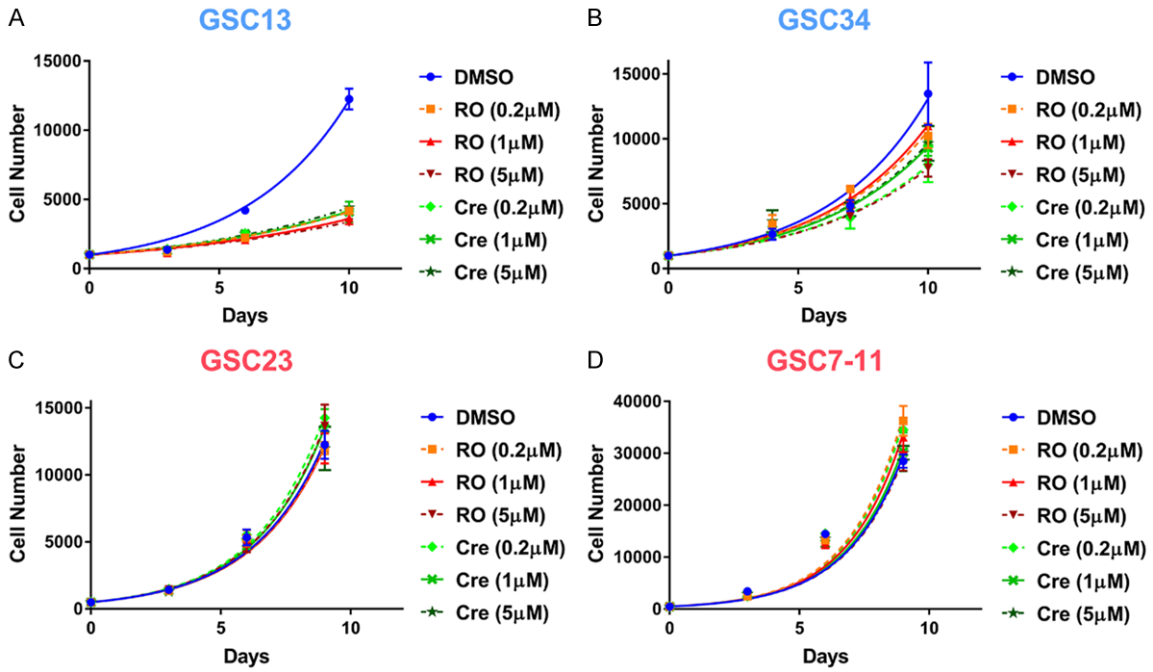


Figure S5. Effect of GSIs on GSC growth. The effect of GSIs (RO and crenigacestat) on GSC growth was tested in three doses (0.2 mM, 1 mM, and 5 mM). A, B. GSIs inhibited GSC13 (wt-p53) growth, but the effect was minimal in GSC34 (wt-p53). C, D. GSIs did not affect growth in mut-p53 GSCs (GSC23 and GSC7-11). n = 3. Blue font: wt-p53 cells. Red font: mut-p53 cells.

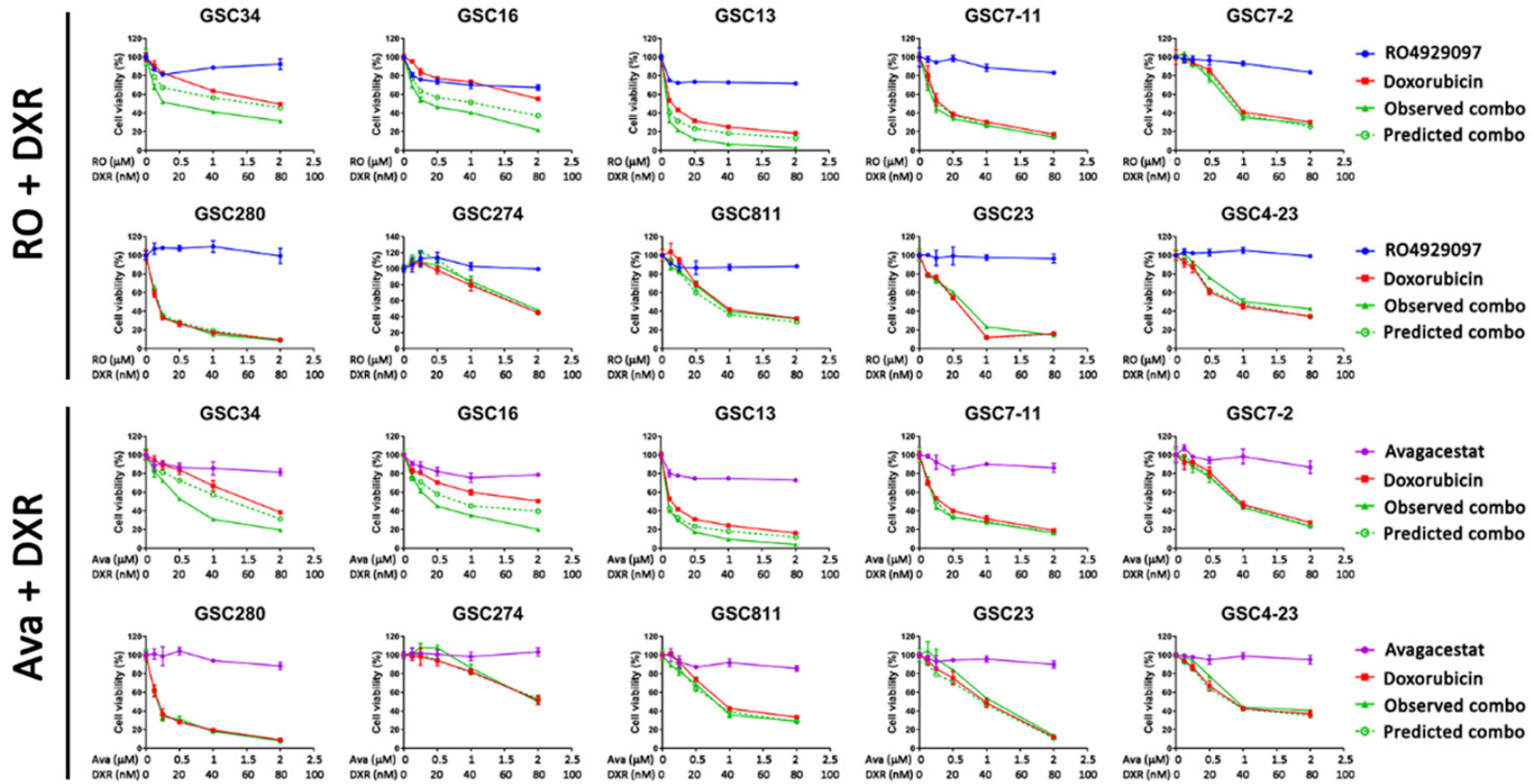


Figure S6. Screening group. The effect of GSI (RO or avagacestat) and doxorubicin treatment (5 days) on GSC proliferation. n = 3.

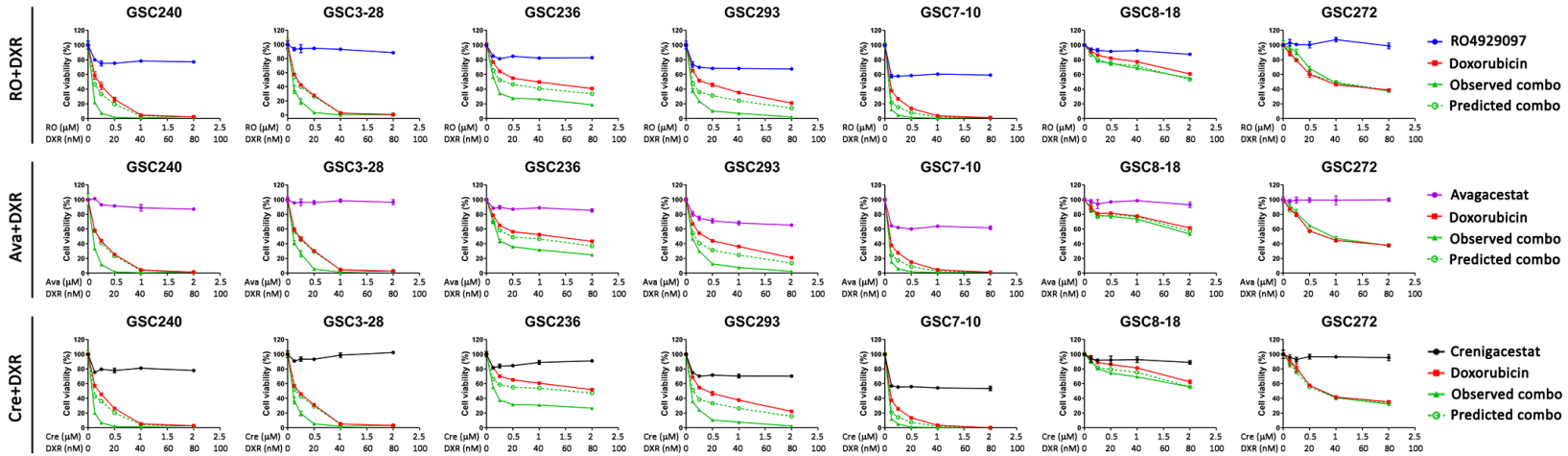
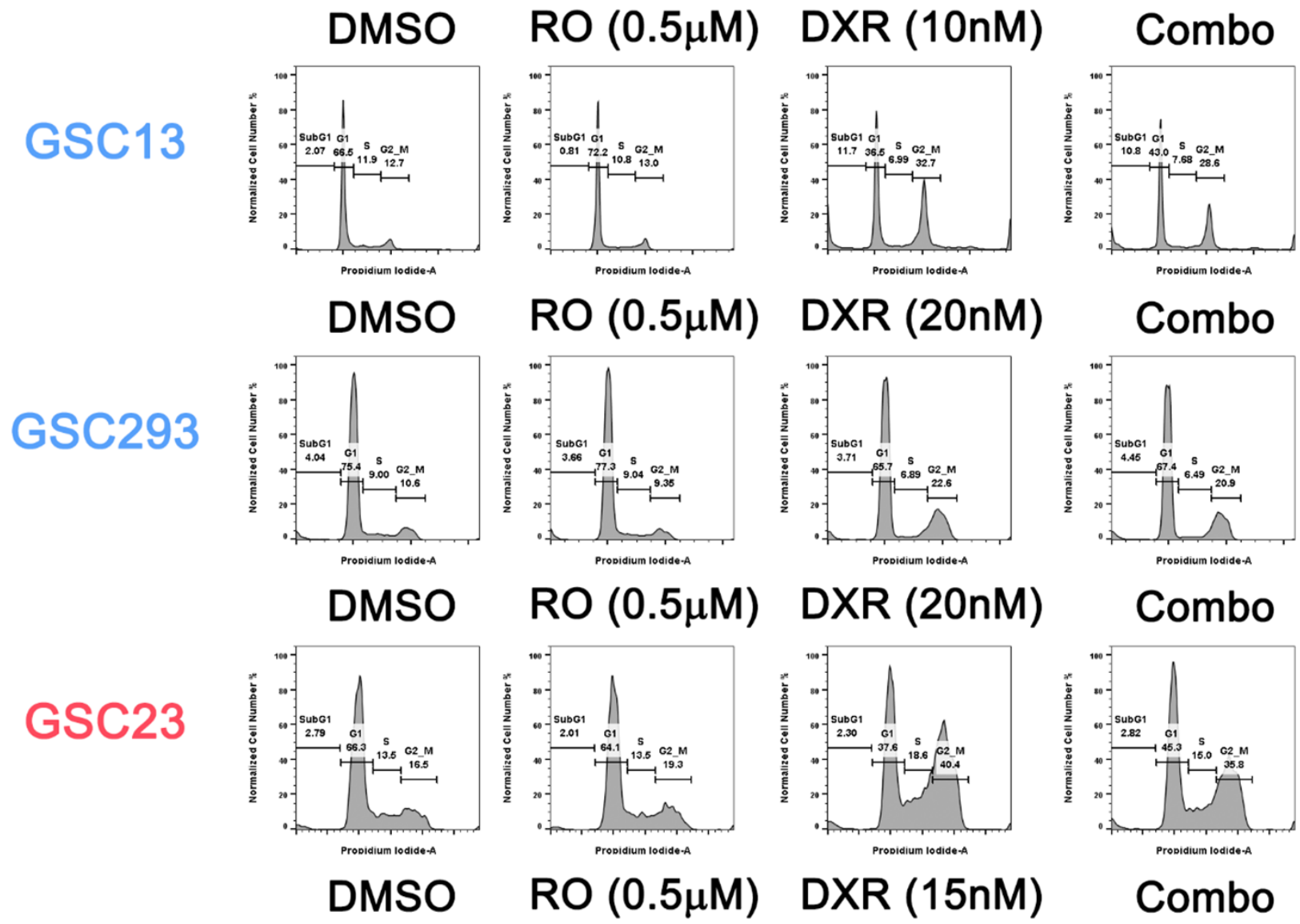


Figure S7. Validation group. The effect of GSI (RO, avagacestat, or crenigacestat) and doxorubicin treatment (5 days) on GSC proliferation. n = 3.


DMSO
RO (0.5μM)
DXR (10nM)
Combo
GSC13
SubG1 2.07 G1 66.5 S 11.9 G2_M 12.7
SubG1 0.81 G1 72.2 S 10.8 G2_M 13.0
SubG1 11.7 G1 36.5 S 6.99 G2_M 32.7
SubG1 10.8 G1 43.0 S 7.68 G2_M 28.6
DMSO
RO (0.5μM)
DXR (20nM)
Combo
GSC293
SubG1 4.04 G1 75.4 S 9.00 G2_M 10.6
SubG1 3.66 G1 77.3 S 9.04 G2_M 9.35
SubG1 3.71 G1 65.7 S 6.89 G2_M 22.6
SubG1 4.45 G1 67.4 S 6.49 G2_M 20.9
DMSO
RO (0.5μM)
DXR (20nM)
Combo
GSC23
SubG1 2.79 G1 66.3 S 13.5 G2_M 16.5
SubG1 2.01 G1 64.1 S 13.5 G2_M 19.3
SubG1 2.30 G1 37.6 S 18.6 G2_M 40.4
SubG1 2.82 G1 45.3 S 15.0 G2_M 35.8
DMSO
RO (0.5μM)
DXR (15nM)
Combo
Normalized Cell Number %
Propidium Iodide-A

GSC7-11

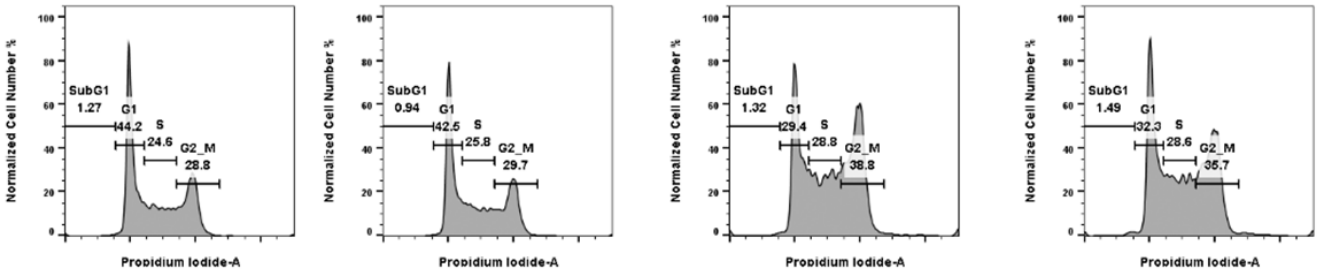
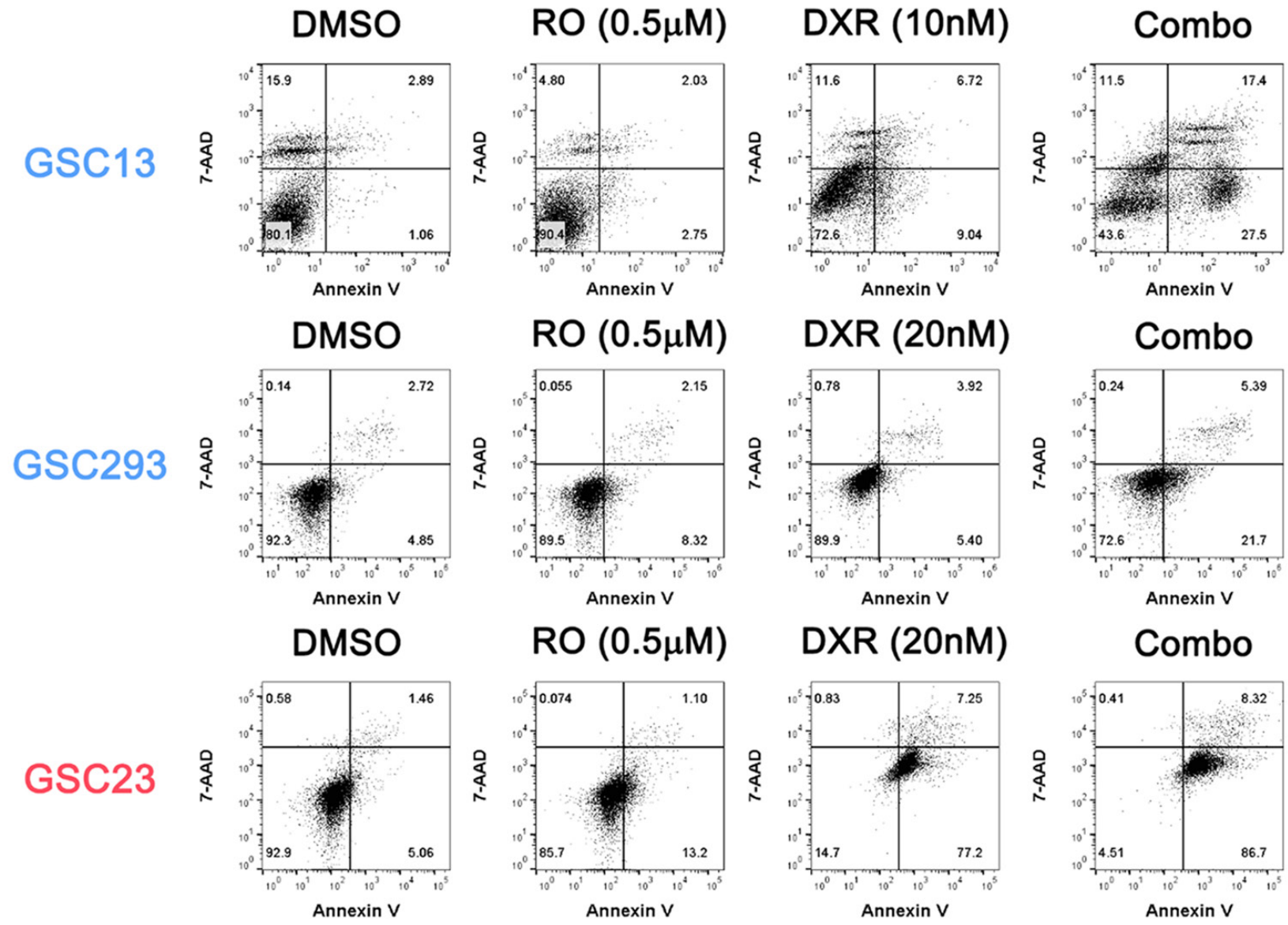


Figure S8. GSI and doxorubicin combination did not induce additional cell cycle arrest. GSCs were treated for 24 hours. Doxorubicin alone induced G2/M arrest in all four GSCs, while RO treatment did not facilitate doxorubicin to induce more cell cycle arrest. Doxorubicin concentration for apoptosis detection was determined by IC50 for each GSC. n = 3. Blue font: wt-p53 cells. Red font: mut-p53 cells.


DMSO
RO (0.5μM)
DXR (10nM)
Combo
GSC13
15.9
2.89
80.1
1.06
4.80
2.03
90.4
2.75
11.6
6.72
72.6
9.04
11.5
17.4
43.6
27.5
DMSO
RO (0.5μM)
DXR (20nM)
Combo
GSC293
0.14
2.72
92.3
4.85
0.055
2.15
89.5
8.32
0.78
3.92
89.9
5.40
0.24
5.39
72.6
21.7
DMSO
RO (0.5μM)
DXR (20nM)
Combo
GSC23
0.58
1.46
92.9
5.06
0.074
1.10
85.7
13.2
0.83
7.25
14.7
77.2
0.41
8.32
4.51
86.7
7-AAD
Annexin V

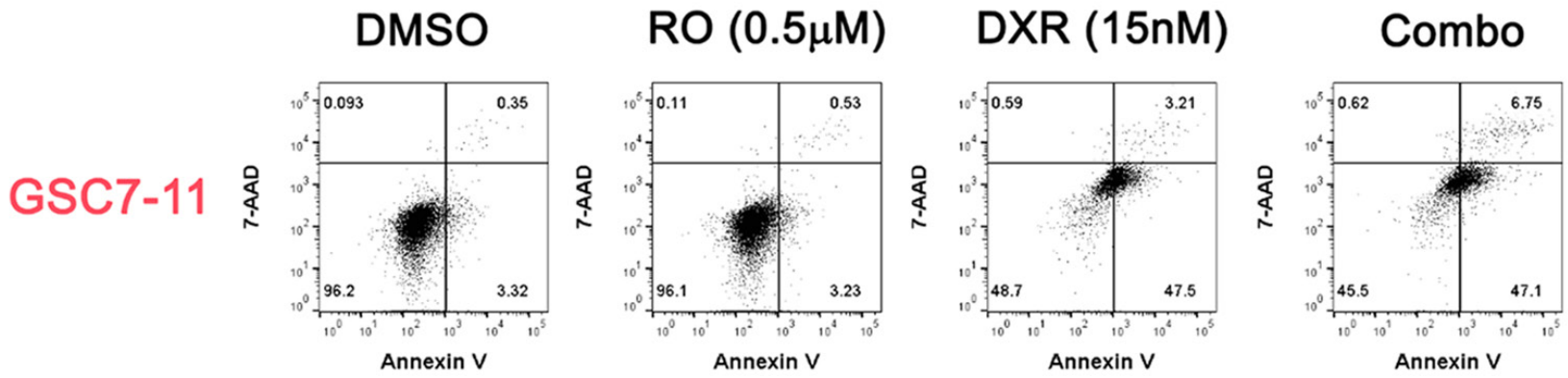


Figure S9. GSI and doxorubicin combination induced more apoptosis in wt-p53 GSCs. GSCs were treated for 24 hours and then stained with FITC-annexin V/7-AAD. Doxorubicin concentration for apoptosis detection was determined by IC50 for each GSC. n = 3. Blue font: wt-p53 cells. Red font: mut-p53 cells.

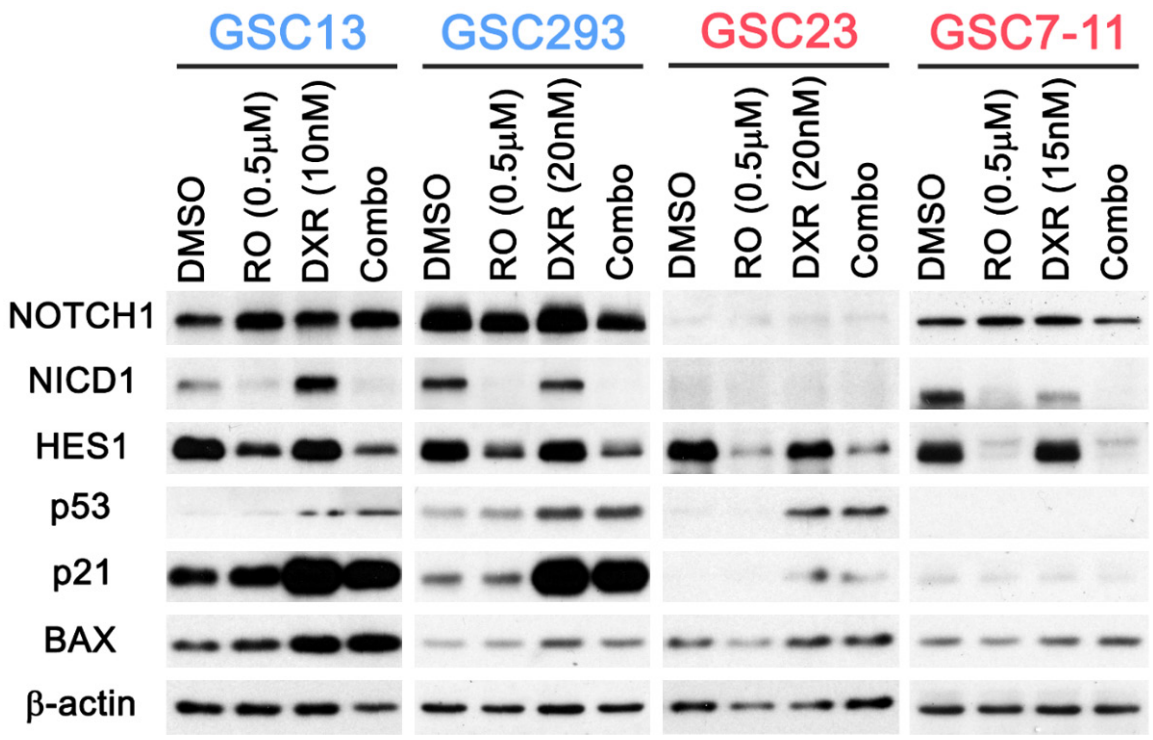


Figure S10. Effect of GSI and doxorubicin combination on NOTCH1 and p53 pathway expression. GSCs were treated for 24 hours and then subjected to Western blot analysis. Doxorubicin concentration for apoptosis detection was determined by IC50 for each GSC. n = 3. Blue font: wt-p53 cells. Red font: mut-p53 cells.