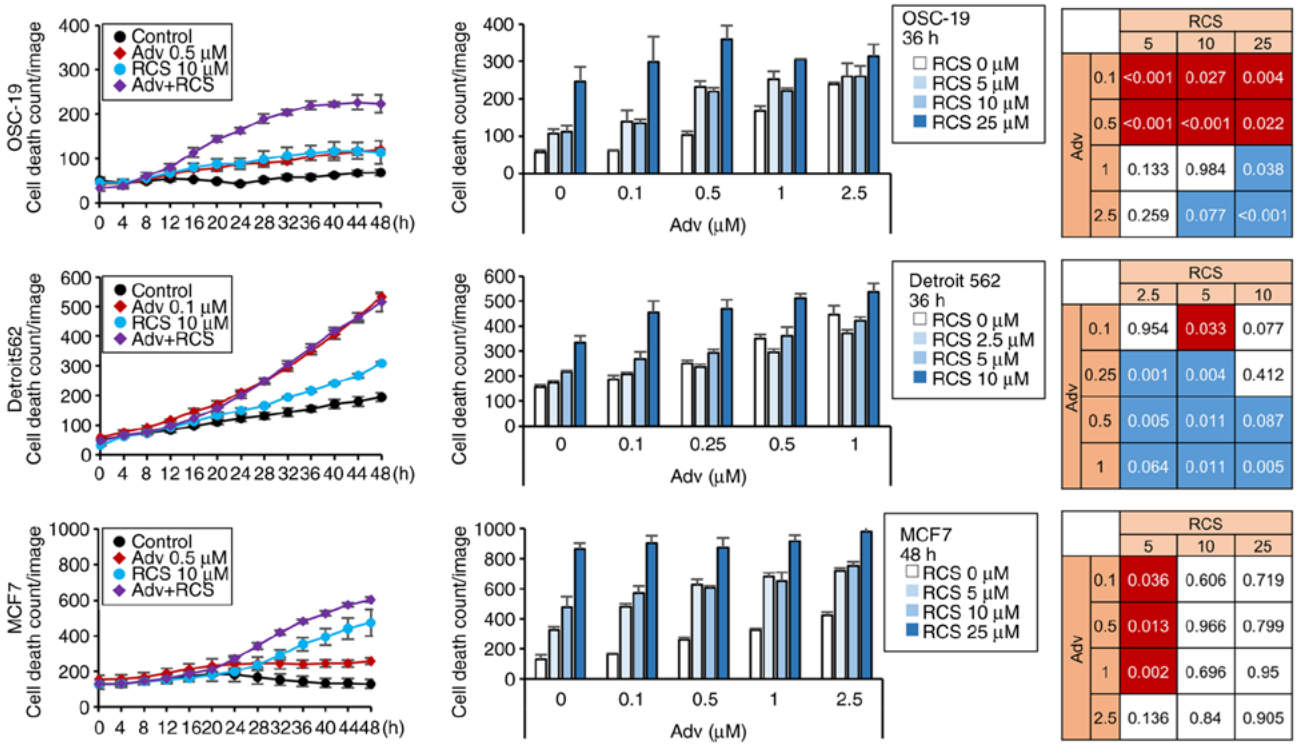
Figure S1. Ricolinostat enhancesadavosertib-induced cytotoxicity in OSC-19 but not or very weakly in Detroit562 and MCF7 cells. OSC-19, Detroit562, and MCF7 cells were treated with Adv in combination with RCS for up to 48 h. Cells were monitored using IncuCyte live cell imaging system and dead cell number was assessed using PI staining. The synergistic effect on cell death in the combination treatment was assessed and is summarized in the right panels. Representative data of three independent experiments are shown. n=3; bar, mean ± SD. Adv, adavosertib; RCS, ricolinostat.




| Adv \ RCS | 5 | 10 | 25 |
|---|---|---|---|
| 0.1 | <0.001 | 0.027 | 0.004 |
| 0.5 | <0.001 | <0.001 | 0.022 |
| 1 | 0.133 | 0.984 | 0.038 |
| 2.5 | 0.259 | 0.077 | <0.001 |

| Adv \ RCS | 2.5 | 5 | 10 |
|---|---|---|---|
| 0.1 | 0.954 | 0.033 | 0.077 |
| 0.25 | 0.001 | 0.004 | 0.412 |
| 0.5 | 0.005 | 0.011 | 0.087 |
| 1 | 0.064 | 0.011 | 0.005 |

| Adv \ RCS | 5 | 10 | 25 |
|---|---|---|---|
| 0.1 | 0.036 | 0.606 | 0.719 |
| 0.5 | 0.013 | 0.966 | 0.799 |
| 1 | 0.002 | 0.696 | 0.95 |
| 2.5 | 0.136 | 0.84 | 0.905 |

Figure S2. TP53-KO in MCF7 cells enhances synergistic cell death by combined treatment of adavosertib andricolinostat. (A) TP53-KO in MCF7 cells was confirmed by western blotting. Wild-type and TP53-KO MCF7 cells were treated with 1 $\mu$M DOX for 24 h. (B) TP53-WT and TP53-KO MCF7 cells were treated with Adv in combination with RCS for up to 48 h. Cells were monitored using IncuCyte live cell imaging system, and dead cell number was assessed by PI staining. Dose-dependent and time-dependent cell death numbers are shown. The synergistic effect on cell death in combination treatment was assessed and is summarized in the right panel. Representative data of three independent experiments are shown. n=4; bar, mean ± SD. KO, knockdown; Adv, adavosertib; RCS, ricolinostat.

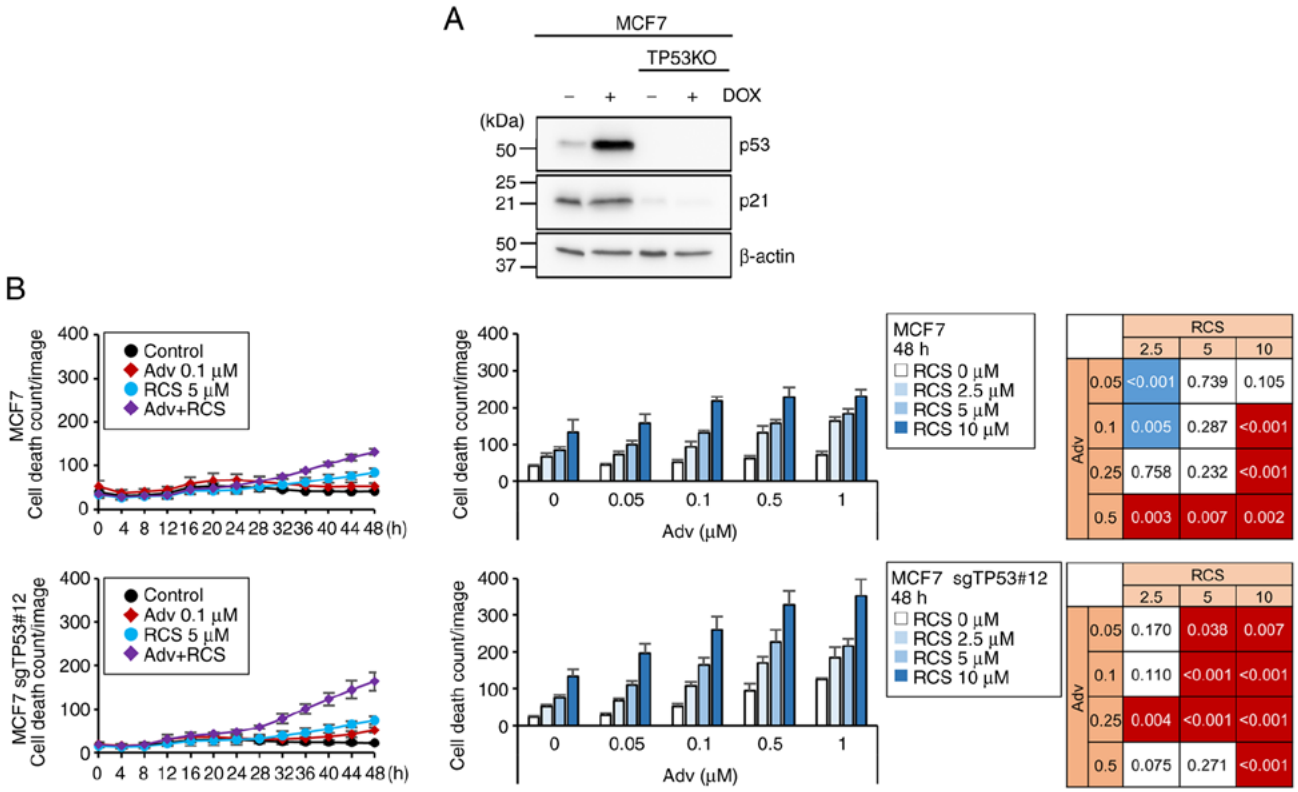


Figure S3. Combined treatment of adavosertiband ricolinostatdoes not increase DNA damage in Detroit562 cells. (A) Non-functional p53 in Detroit562 was confirmed by western blotting. A549 cells and Detroit562 cells were treated with 1 $\mu$M DOX for 24 h. Induction of p21 and MDM2 was confirmed. β-actin was loaded as control. (B) DDR-related protein expression in Detroit562 cells was confirmed via western blotting. Cells were treated with Adv (0.1 $\mu$M), RCS (10 $\mu$M), and Adv + RCS for 24 h. Adv, adavosertib; RCS, ricolinostat; DDR, DNA damage response; MDM2, murine double minute 2; Chk, checkpoint kinase; CDK1, cyclin-dependent kinase 1.