Supplementary Figures

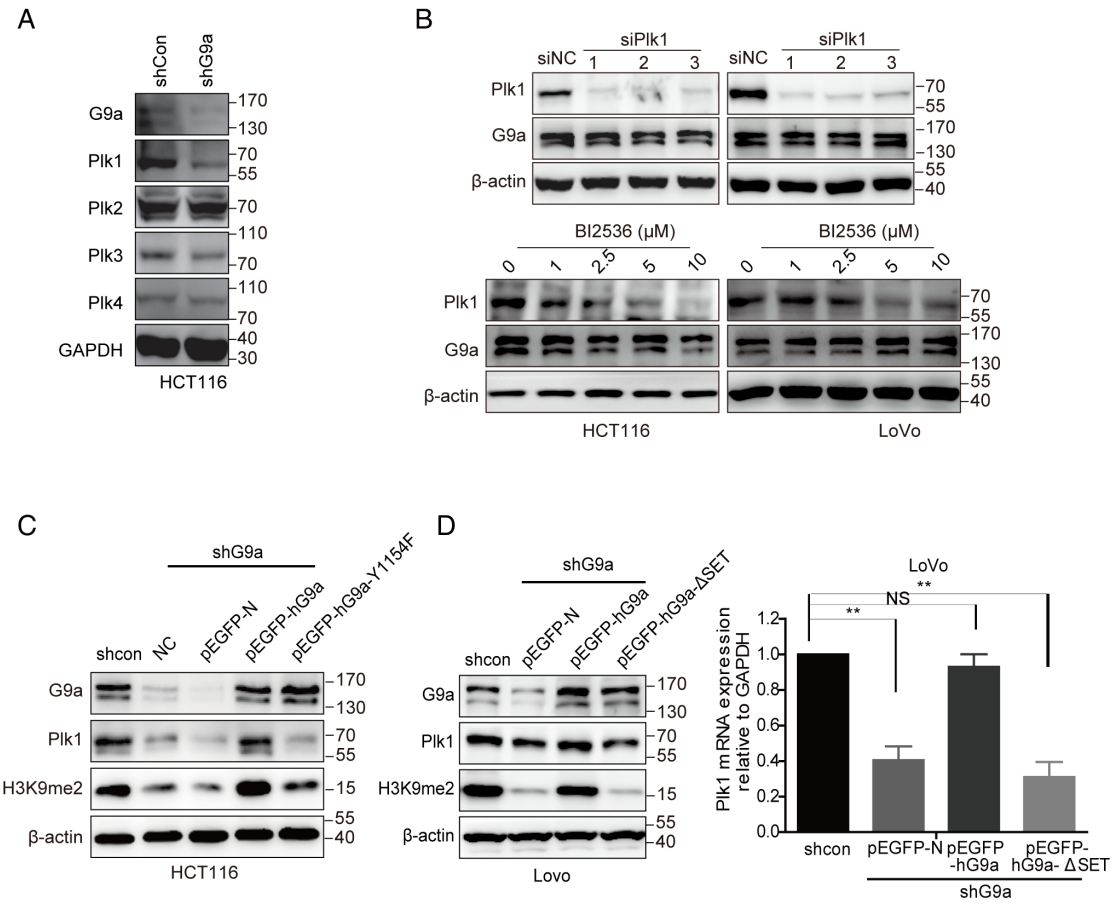

Figure S1. G9a regulates Plk1 expression. (A) Levels of Plk family proteins such as Plk1, Plk2, Plk3, and Plk4 in HCT116 cells. (B) Plk1 knockdown did not have any obvious effect on G9a protein level in HCT116 or LoVo cells. Treatment with Plk1 inhibitor BI2536 did not affect G9a expression at the effective concentration. (C) Plk1 expressions in G9a-knockdown, WT G9a-expressing, and G9a Y1154F mutant rescued HCT116 cells. (D) Relative mRNA and protein level of Plk1 in G9a-knockdown, WT G9a-expressing, and G9a SET domain-deleted rescued LoVo cells. All data are representative of at least three independent experiments and are expressed as mean ± SD; *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$.

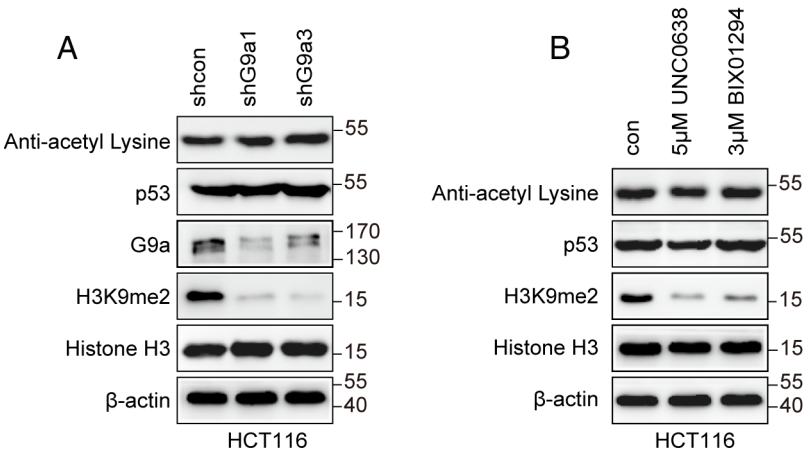

Figure S2. Pan-acetylation (A, B) of p53 after treatment with UNC0638 or BIX01294 or knockdown of G9a in HCT116 p53$^{+/+}$ or LoVo cells.

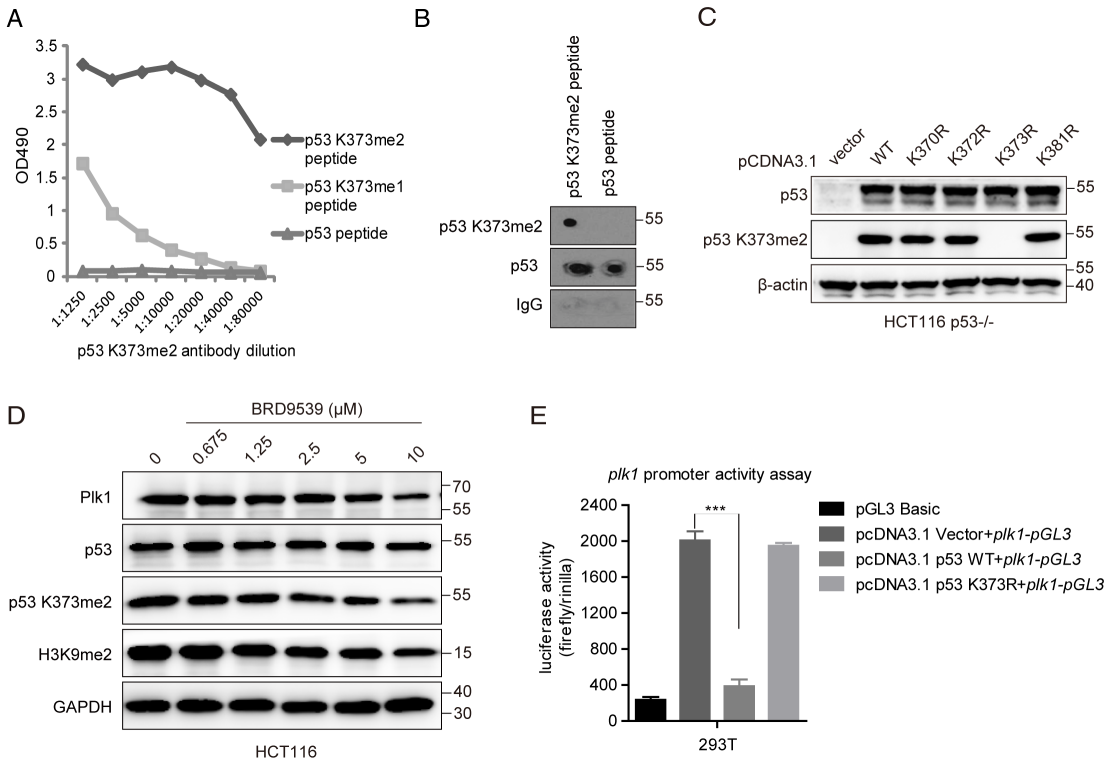

Figure S3. p53K373me2 antibody specificity assay. (A) To titrate the p53K373me2-mAb, seven dilutions of the antibody were assayed for binding to purified p53, p53K373me1 of p53K373me2 peptides by direct ELISA. (B) Monoclonal antibody against p53K373me2 was prepared and examined by performing the dot assay. (C) Western blotting analysis detected the p53 K373me2 levels in the whole-cell extracts of cells transfected with plasmids expressing WT p53, p53K370R, p53K372R, p53K373R or p53K381R in HCT116 $p53^{-/-}$. Protein levels of Plk1, p53, p53K373me2, and H3K9me2 in HCT116 cells treated with different concentrations of G9a SAM-competitive inhibitor BRD9539 (D) (E) The effects of the p53 wild-type and the K373R mutant on PlK1 promoter activity were tested. All data are representative of at least three independent experiments and are expressed as mean ± SD; $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$.

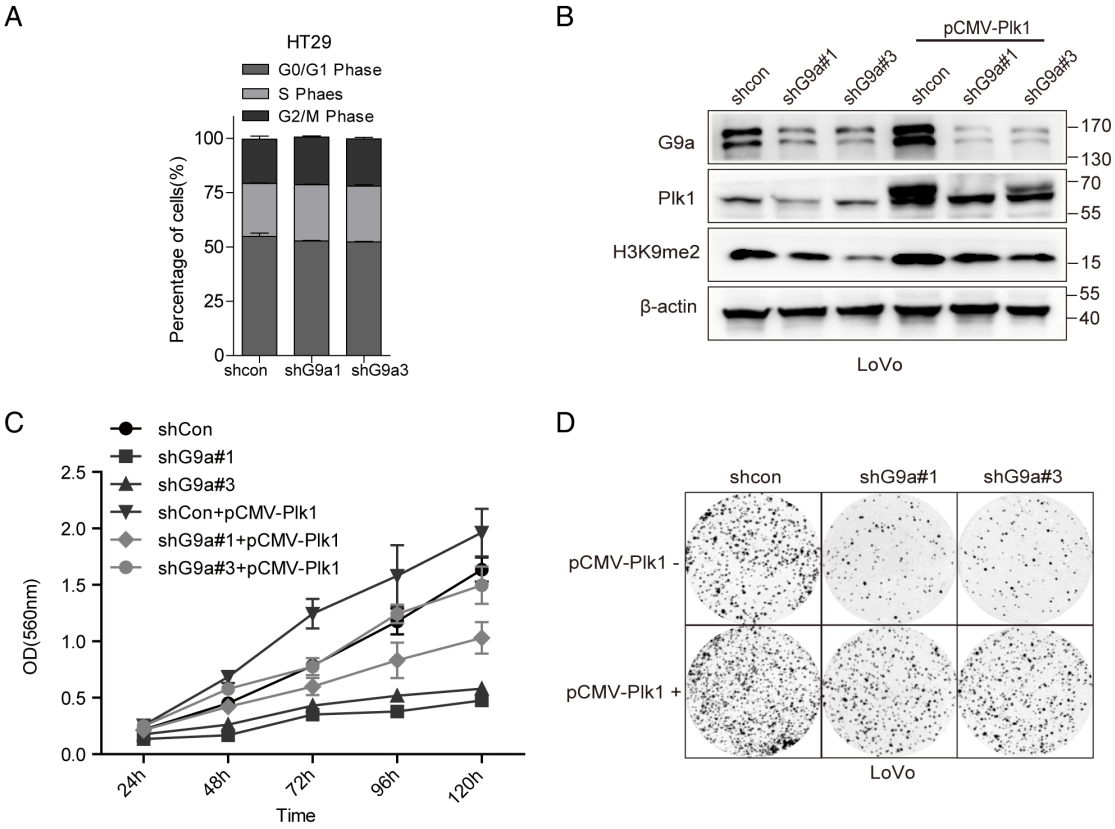

Figure S4. G9a regulates CRC growth by regulating Plk1 (A) G9a knockdown did not affect cell cycle progression in HT29 cells. Plk1 restoration (B) promoted the (C) proliferation of and (D) colony formation in LoVo cells. All data are representative of at least three independent experiments and are expressed as mean ± SD. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$.

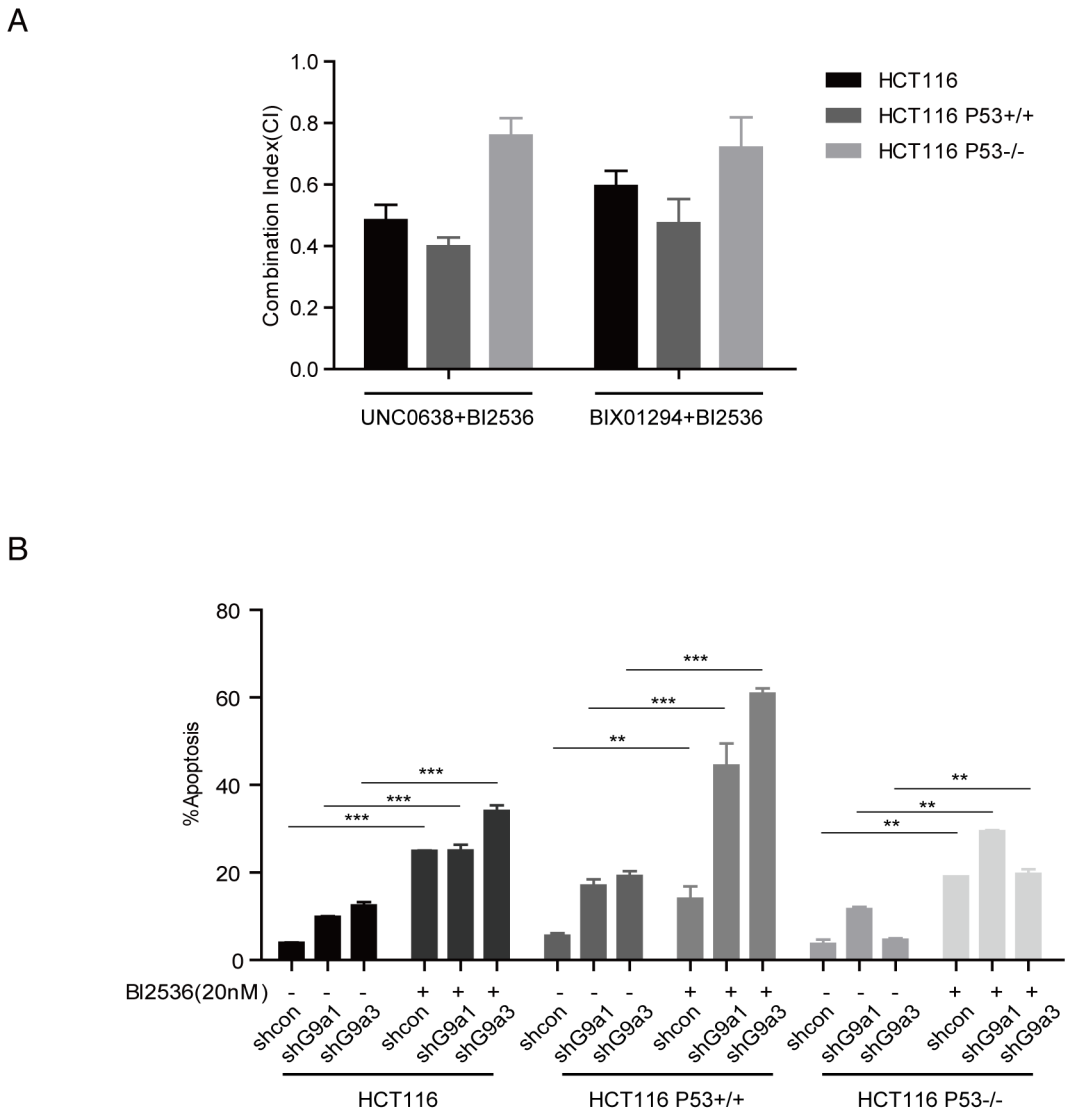

Figure S5. G9a and Plk1 inhibitors exert synergistic effects in CRC cells. (A) Effects of combination therapy with BI2536 and G9a inhibitor UNC0638 or BIX01294 were determined by calculating CI by using the CalcuSyn program. A CI of <0.8 indicates a synergistic effect. (B) Treatment with Plk1 inhibitor BI2536 increased the apoptosis of G9a-knockdown HCT116 $p53^{+/+}$ and HCT116 $p53^{-/-}$ cells compared with that of sh-control cells. All data are representative of at least three independent experiments and are expressed as mean ± SD; $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$.

Supplementary Table 1: Primer sequences used for amplification

Primers for genomic DNA amplification (reporter gene assay)

1	***plk1* sense**	5'-CGACGCGTATGGTGAAACCTCTTCTCTACTAAA-3'
2	***plk1* antisense**	5'-CCGCTCGAGCGCACCGCTCCGCTCCTCCCCGAAT-3'

Primers for cDNA fragment amplification (RT-PCR) assay

3	**G9a up**	5'-GGAGCCACGAGGGGTGTCCA-3'
4	**G9a down**	5'-CGGCATTGCAGCCTGACAGC-3'
5	**Plk1 up**	5'-GGATCACACCAAGCTCATCTTG-3'
6	**Plk1 down**	5'-CCCGCTTCTCGTCGATGT-3'
7	**GAPDH up**	5'-TGCTGGCGCTGAGTACGTCG-3'
8	**GAPDH down**	5'-GGTGGTGCAGGAGGCATTGCT-3'

Primers for DNA fragment amplification (chromatin immunoprecipitation assay)

9	**P53 binding R2**	5'-GAATTTCCCAGGGCCTAAAATAG-3'
10	**P53 binding F2**	5'-TGCTAGGCATTCTTCCAACCT T-3'