Figure.S1 related to Figure 2

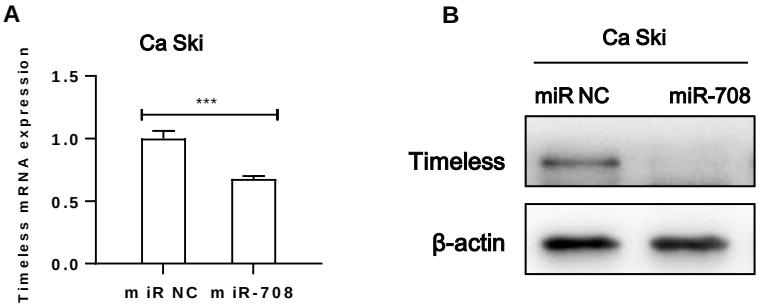

Fig. S1 related to Fig. 2. Timeless is a direct target gene of miR-708. (A) Cervical cancer cells were transfected with indicated miRNAs, and Timeless mRNA expression was analyzed by qPCR 48 h after transfection. The β-actin mRNA was used as an endogenous control. (B) Cervical cancer cells were transfected with indicated miRNAs, and expression level of Timeless was analyzed by Western blotting 48 h after transfection. (***P < 0.001)

Figure.S2 related to Figure 3

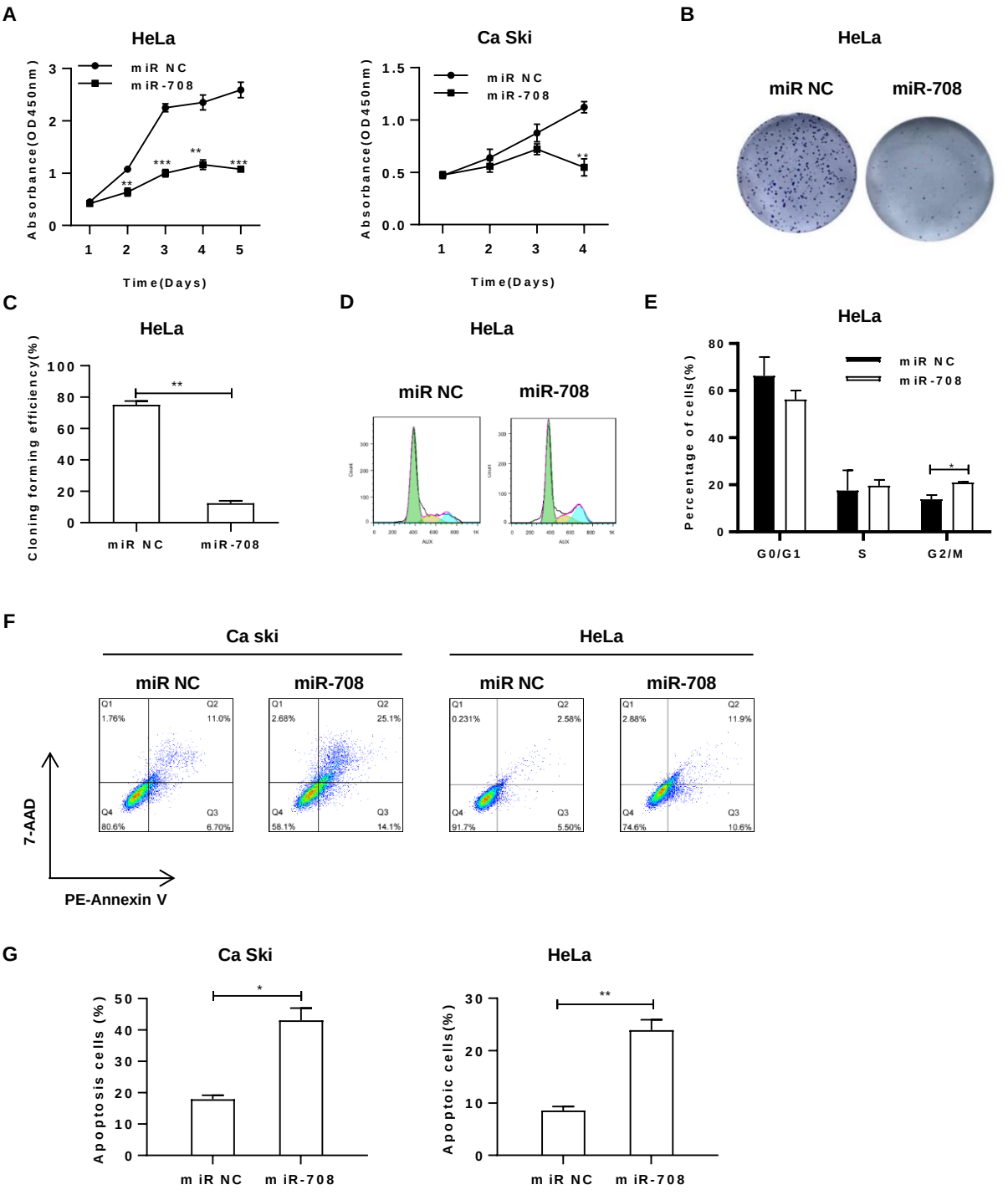

Fig. S2 related to Fig. 3. MiR-708 suppresses proliferation of cervical cancer cells and promotes apoptosis. (A) After transfected with indicated miRNA, the viability of cells was analyzed by Cell Counting Kit-8 (CCK-8) assay at indicated time points. (B) After transfected with indicated miRNA, cells were plated in 6-well plates at density of 500 cells/plate, and incubated for 10-12 days. Cell colonies were visualized by crystal violet staining. The representative images were shown. (C) The quantitative analysis of three independent colony formation assays. (D) 48 h after transfected with indicated miRNA, cell cycle was determined by flow cytometry analysis. (E) Quantitative analysis was represented as mean ± standard deviation (SD) of cell percentage in each cycle in three independent experiments. (F) 48 h after transfected with indicated miRNA, cell apoptosis was determined by PE-Annexin V/7-AAD staining and flow cytometry. (G) The quantitative results were represented as the mean ± standard deviation (SD) of three independent experiments. (*P < 0.05, **P < 0.01, ***P < 0.001)

Figure.S3 related to Figure 4

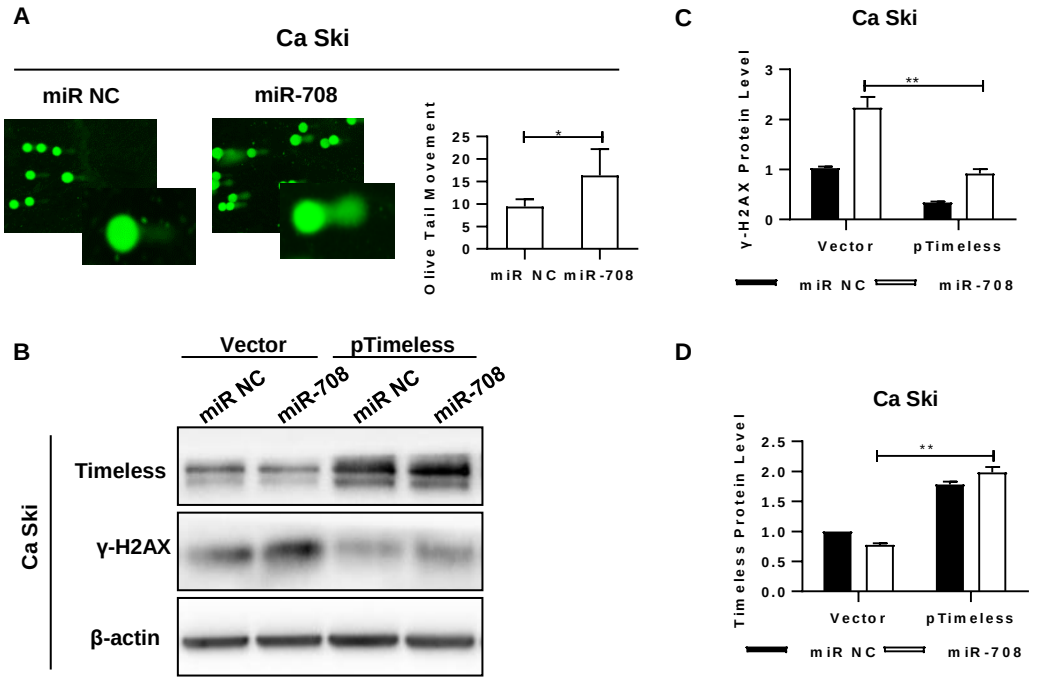

Fig. S3 related to Fig. 4. miR-708 induces DNA damage and impairs activation of ATR signaling pathway. (A) 72 h after transfected with indicated miRNA, cells were subjected to the Comet assay and stained by Vista Green DNA Dye. The comet images were analyzed by CASP software, and the olive tail moment (OTM) was considered for the analysis of parameters. Approximately 100 cells in each group were counted. Quantitative results were represented as the mean ± standard deviation (SD) of three independent experiments. (B) Ca Ski cells were transfected with indicated miRNA and pIRES2-EGFP-NC (Vector) or pIRES2-EGFP-Timeless (pTimeless). Western blotting was conducted 72 h after transfection. The quantitative results of γ-H2AX (C) and Timeless (D) were represented as the mean ± SD of three independent experiments. (*P < 0.05, **P < 0.01)

Table S1 Correlation of miR-708 expression by qPCR with clinicopathological parameters in cervical cancer patients.

		miR-708 expression (%)		
Variable	**Cases（n）**	**Low**	**High**	**P-value**
Age（years）				
<45	6	5 (83.33)	1 (16.67)	1.000
≥45	14	12 (85.71)	2 (14.29)	
Tumor size				
≤4cm	19	16 (84.21)	3 (15.79)	1.000
>4cm	1	1 (100.00)	0 (0.00)	
Histological type				
Squamous cell carcinoma	18	15 (83.33)	3(16.67)	1.000
Adenocarcinoma	2	2 (100.00)	0 (0.00)	
FIGO stage				
≤IB	10	10 (100.00)	0 (0.00)	0.211
>IB	10	7 (70.00)	3 (30.00)	
Lymph node metastasis				
No	14	11 (78.57)	3 (21.43)	0.521
Yes	6	6 (100.00)	0 (0.00)	

Abbreviations: FIGO staging: the Cervical Cancer Staging System of International Federation of Gynecology and Obstetrics in 2018.