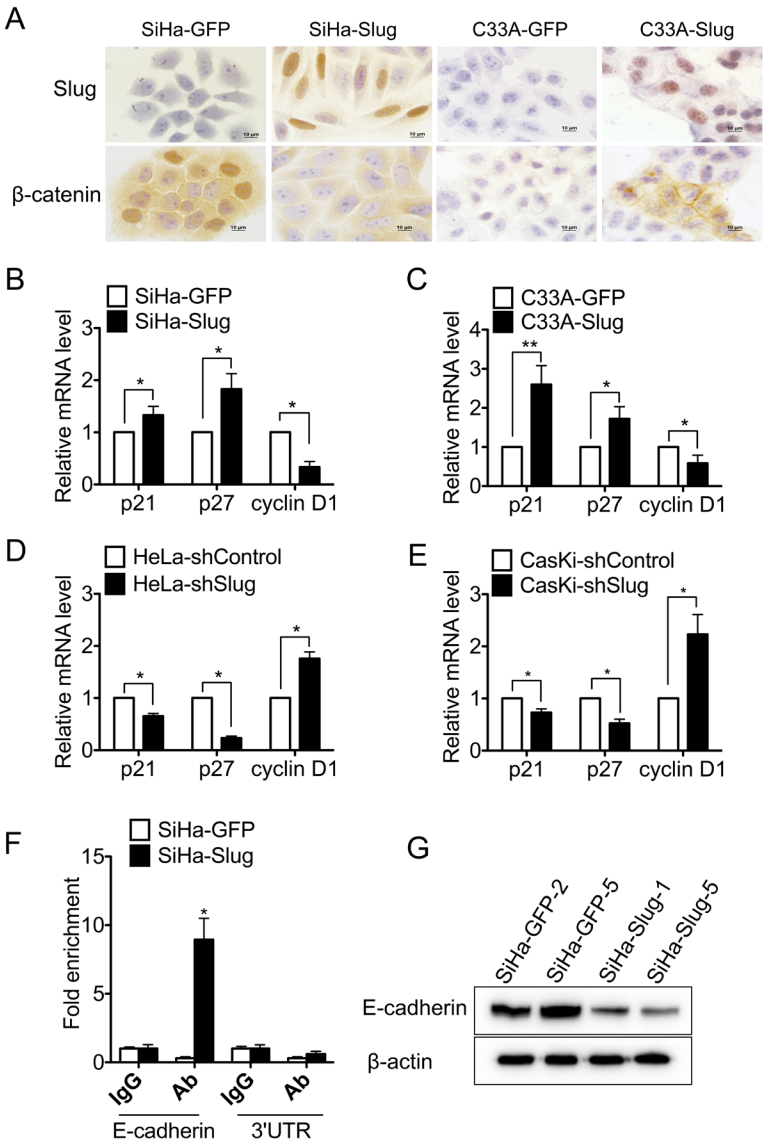
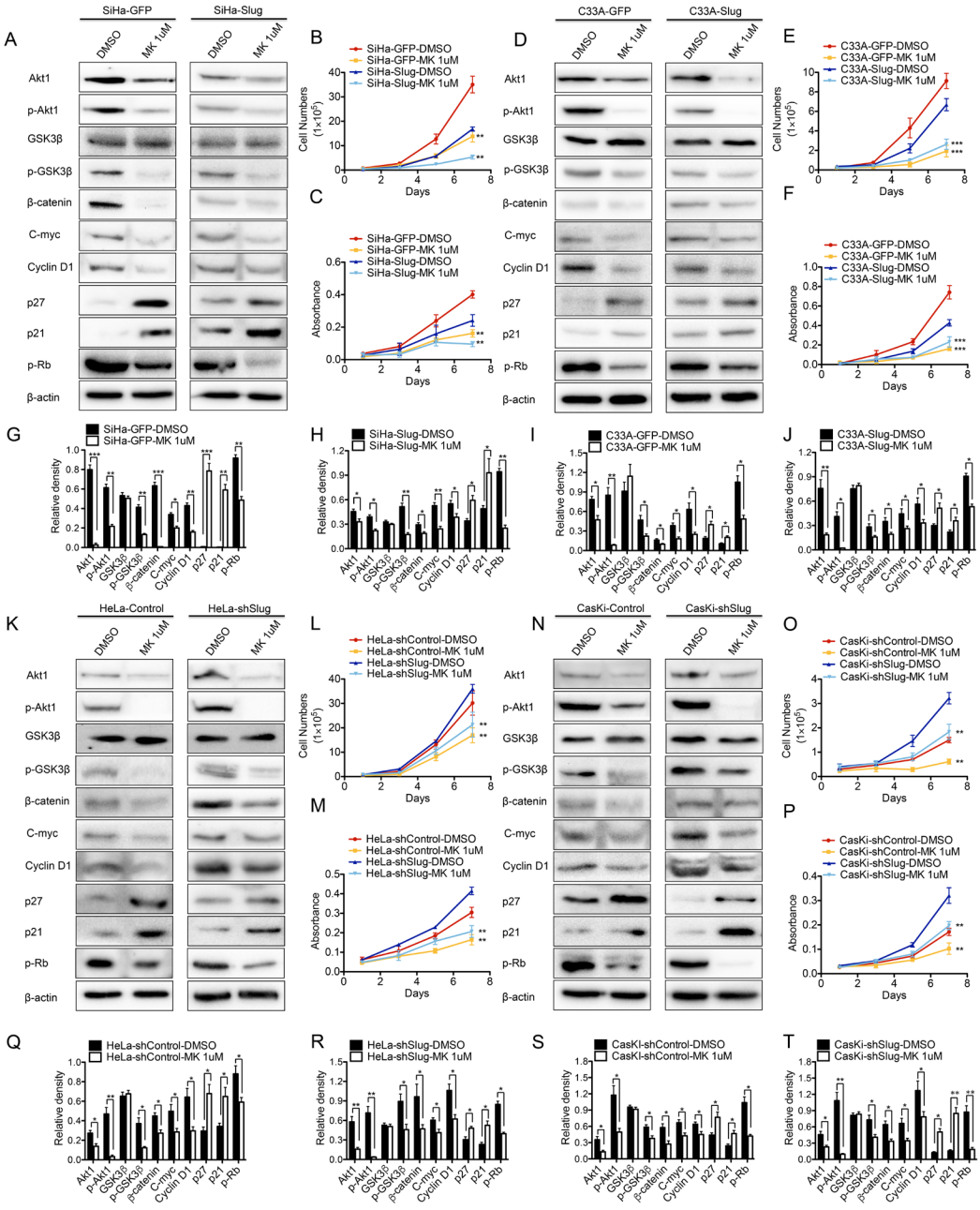
Slug inhibits the proliferation and tumor formation of human cervical cancer cells by up-regulating the p21/p27 proteins and down-regulating the activity of the Wnt/β-catenin signaling pathway via the trans-suppression Akt1/p-Akt1 expression

Supplementary Materials



Supplementary Figure S1: (A) IHC detection of β-catenin and slug in SiHa-GFP, SiHa-Slug, C33A-GFP and C33A-Slug cells. (**B**) The expression of p21, p27 and cyclinD1 in SiHa-GFP and SiHa-Slug cells was detected by quantitative real-time–PCR. (**C**) The expression of p21, p27 and cyclinD1 in C33A-GFP and C33A-Slug cells was detected by quantitative real-time–PCR. (**D**) The expression of p21, p27 and cyclinD1 in HeLa-shControl and HeLa-shSlug cells was detected by quantitative real-time–PCR. (**E**) The expression of p21, p27 and cyclinD1 in Caski-shControl and Caski- shSlug cells was detected by quantitative real-time–PCR. (**F**) A quantitative CHIP assay of the E-cadherin promoter region in SiHa-Slug and SiHa-GFP cells is shown. (**G**) The expression of E-cadherin in SiHa-Slug and SiHa-GFP cells was detected by western blotting. The data were shown as the mean ± SD of three independent experiments. *$p < 0.05$, **$p < 0.01$ *vs*. control using One-Way ANOVA.



Supplementary Figure S2: Blockage of the Akt1/p-Akt1 suppresses cell proliferation in the slug-modified cervical cancer cells by MK-2206. (**A**) The expression of Akt1, p-Akt1, p21, p27, p-Rb, p-GSK3β, GSK3β, β-catenin, c-myc and cyclinD1 in MK-treated SiHa-GFP and SiHa-Slug cells was detected by western blotting, and the quantitative analysis was shown (**G** and **H**). The proliferation and viability of MK-treated SiHa-GFP and SiHa-Slug cells were detected by growth curves (**B**) and MTT assay (**C**). (**D**) The expression of Akt1, p-Akt1, p21, p27, p-Rb, p-GSK3β, GSK3β, β-catenin, c-myc and cyclinD1 in MK-treated C33A-GFP and C33A-Slug cells was detected by western blotting, and the quantitative analysis was shown (**I** and **J**). The proliferation and viability of MK-treated C33A-GFP and C33A-Slug cells were detected by growth curves (**E**) and MTT assay (**F**). (**K**) The expression of Akt1, p-Akt1, p21, p27, p-Rb, p-GSK3β, GSK3β, β-catenin, c-myc and cyclinD1 in MK-treated HeLa-shControl and HeLa-shSlug cells was detected by western blotting, and the quantitative analysis was shown (**Q** and **R**). The proliferation and viability of MK-treated HeLa-shControl and HeLa-shSlug cells were detected by growth curves (**L**) and MTT assay (**M**). (**N**) The expression of Akt1, p-Akt1, p21, p27, p-Rb, p-GSK3β, GSK3β, β-catenin, c-myc and cyclinD1 in MK-treated Caski-shControl and Caski-shSlug cells was detected by western blotting, and the quantitative analysis was shown (**S** and **T**). The proliferation and viability of MK-treated Caski-shControl and Caski- shSlug cells were detected by growth curves (**O**) and MTT assay (**P**). The data were shown as the mean ± SD of three independent experiments. *$p < 0.05$, **$p < 0.01$ *vs*. control using One-Way ANOVA.

Supplementary Table S1: Slug expression levels in different tissue specimen

Specimens	Total	Slug Staining		*P*
		Negative, No. (%)	Positive, No. (%)	
Normal	38	4.667 ± 0.5774 (12.69 ± 0.8083)	33.33 ± 0.5774 (87.72 ± 1.518)	
Cancer *in situ*	24	9.667 ± 1.155 (40.28 ± 4.809)	14.33 ± 1.155 (59.73 ± 4.792)	< 0.05[a]
Carcinoma	52	17.67 ± 1.155 (33.96 ± 2.200)	31.67 ± 1.155 (62.18 ± 2.217)	< 0.05[b]

Abbreviation: Slug

Pearson 2-tailed chi-square test was used to determine the statistical significance of the level of expression of Slug in different tissue specimens.

[a]Normal cervix versus cervical cancer in situ.

[b]Normal cervix versus carcinoma.

Supplementary Table S2: The list of primer sequences that used for luciferase assays in this study

Primer name	Location	F/R	Sequence
P1	–585 ~ –355	F	CGACGCGTCTTTTGTGAGTGTAG
		R	GGAAGATCTTGGCTTAGGTTGACTT
P2	–767 ~ –511	F	CGACGCGTTGGACTTCGGACT
		R	GGAAGATCTTGGCAGCTACACTCAC
P3	–831 ~ –706	F	CGACGCGTTGTCCAGGAGAAAG
		R	GGAAGATCTTGCTGGGTGGACTTG
P4	–886 ~ –744	F	CGACGCGTAGAACTTCTGGCT
		R	GGAAGATCTGGAATGAGTAAGTGG
P5	–918 ~ –846	F	CGACGCGTAACTCTGGAATGG
		R	GGAAGATCTACCCCTTCCTAGCC
P6	–1012 ~ -888	F	CGACGCGTAATAAAAATGCTCC
		R	GGAAGATCTCCATTCCAGAGGC
P7	–1116 ~ –923	F	CGACGCGTATTGGCTGCAGACT
		R	GGAAGATCTCGTGAAAGACAGACTCTTG
P8	–1345 ~ –221	F	CGACGCGTAACCCTTGTGTCAGGT
		R	GGAAGATCTTCTCTGGCCTCAGTTTC

Supplementary Table S3: The list of primer sequences that used for chromatin immunoprecipitation assay (ChIP) in this study

Primer name	Location	F/R	Sequence
P1	–534 ~ –462	F	TCAAAGCCTTCCTGCTCCTT
		R	AAGGAAGTGCGGGAGGAT
P2	–738 ~ –644	F	AGGCTGACCAAGTCC
		R	GAGCAGACACCAGACAG
P3	–824 ~ –717	F	GTGTCCAGGAGAAAGG
		R	ATGGGTGGACTTGGTC
P4	–876 ~ –788	F	AACTTCTGGCTAGGAAGG
		R	GAGTAAGTGGGACACAGAC
P5	–915 ~ –858	F	TGTGGGCCTCTGGAATG
		R	CCTTCCTAGCCAGAAGTTC
P6	–982 ~ –900	F	CAATACTTAGCAGCCTCAGG
		R	TTCCAGAGGCCCACAGTT
P7	–1068 ~ –985	F	CTGCCTCTGTCTGCATCT
		R	TTGGGGGAGCATTTTTAT
3′UTR	Akt1 (3′UTR)	F	CGTTTTTGTGCTGTGGGC
		R	CATTTCCCTACGTGAATCG
E-cadherin	–25 ~ 110	F	CGTCGGAACTGCAAAGC
		R	TATGTGCGGTCGGGTCG