miR-2861 acts as a tumor suppressor via targeting EGFR/AKT2/CCND1 pathway in cervical cancer induced by human papillomavirus virus 16 E6

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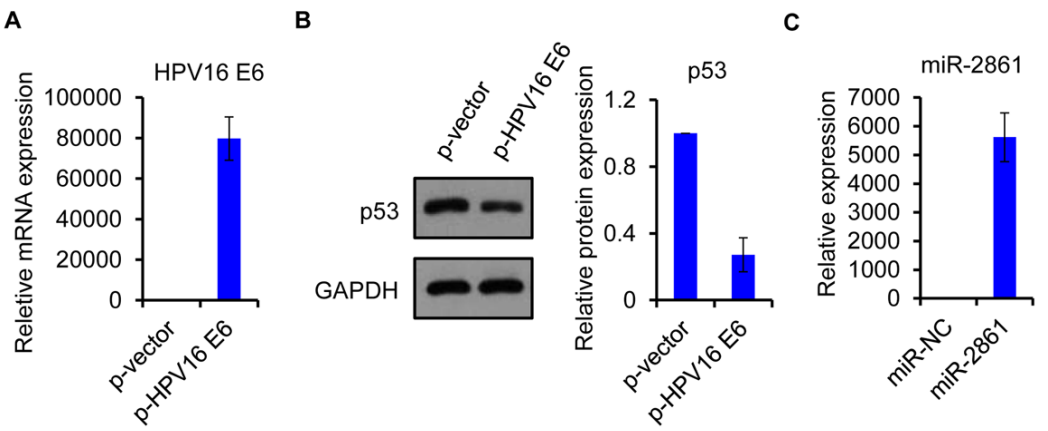

Figure S1. Transfection levels of expression plasmid of HPV16 E6 and miR-2861. (A) At 48h after transfection with HPV16 E6 expression plasmid (2μg) in HEK293T cells, the expression of HPV16 E6 level was determined by qRT-PCR. (B) At 72h after transfection with HPV16 E6 expression plasmid in HEK293T cells, p53 expression level was evaluated by Western blot. (C) At 48h after transfection with miR-2861 mimic (50 nM) in SiHa cells, the expression of miR-2861 was determined by qRT-PCR.

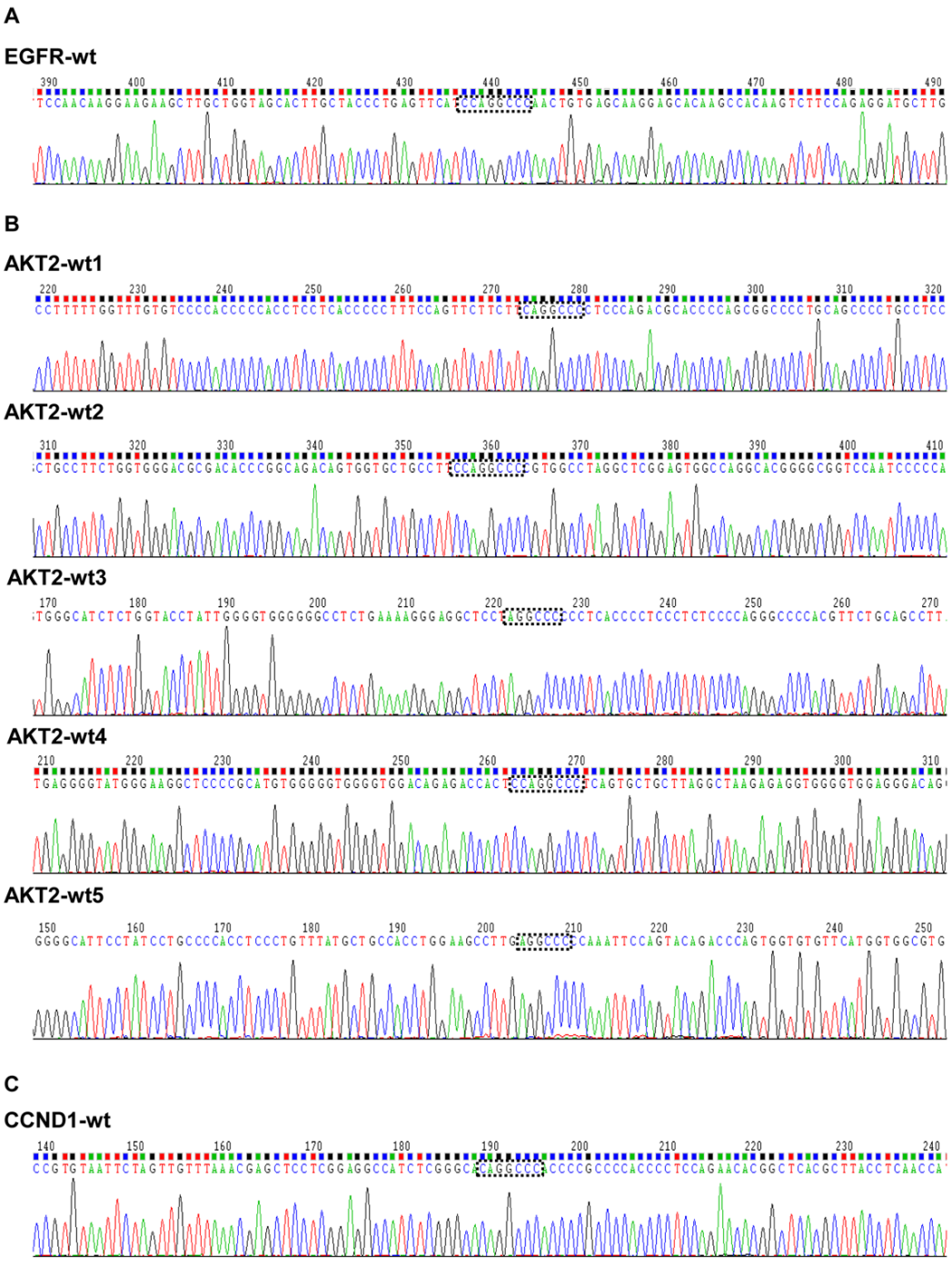

Figure S2. Sequences of EGFR, AKT2, and CCND1 3'UTR containing miR-2861 binding site. (A) Sequence of EGFR 3'UTR containing miR-2861 binding site. (B) Sequences of 5 AKT2 3'UTR wild type plasmids, each of which contains one binding site of miR-2861. (C) Sequence of CCND1 3'UTR containing miR-2861 binding site. Blank box indicates the binding site of miR-2861.

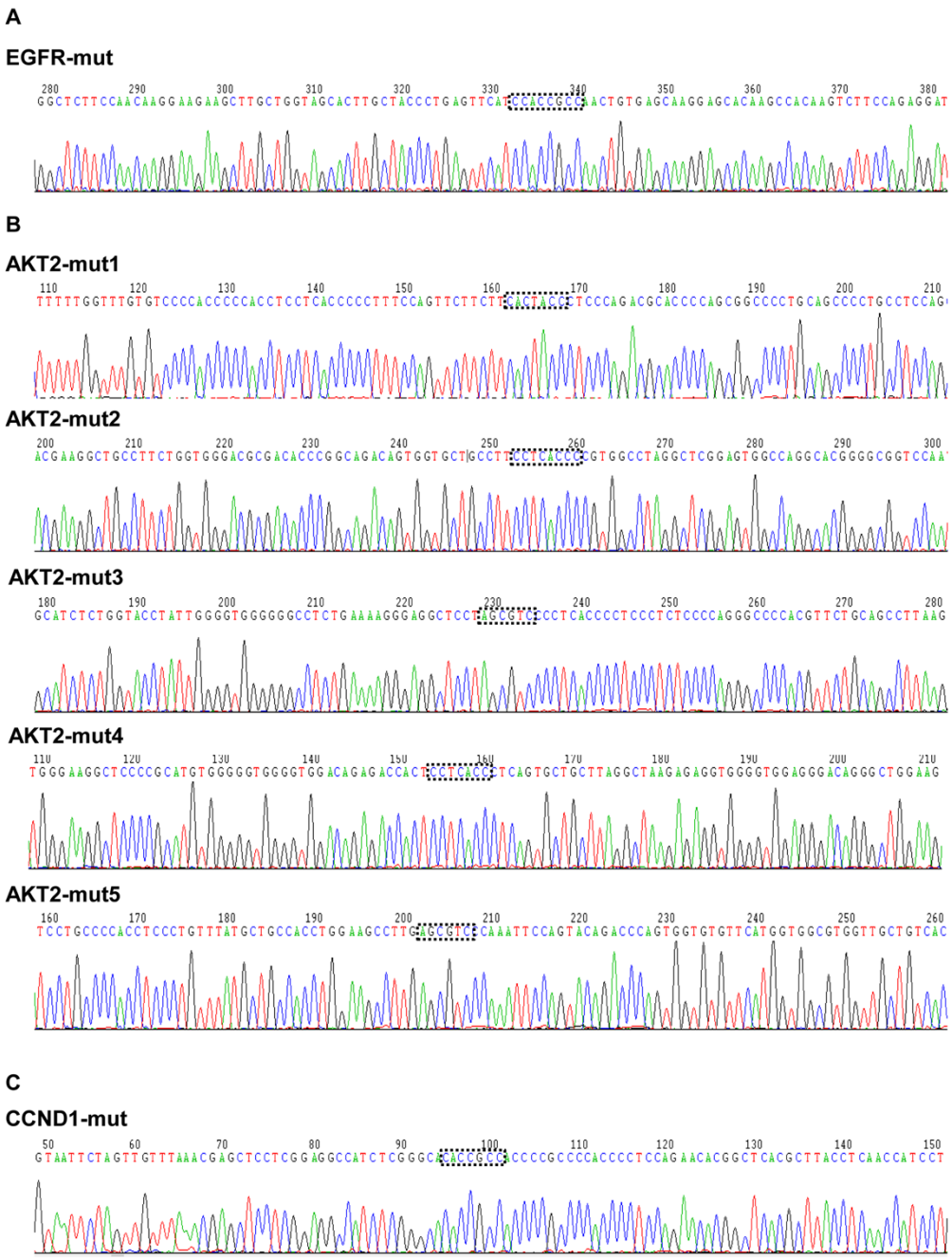

Figure S3. Sequences of mutant EGFR, AKT2, and CCND1 3'UTR. (A-C) The sequences of miR-2861 binding sites on EGFR, AKT2, and CCND1 3'UTR are mutant. Blank box indicates the mutant binding site of miR-2861.

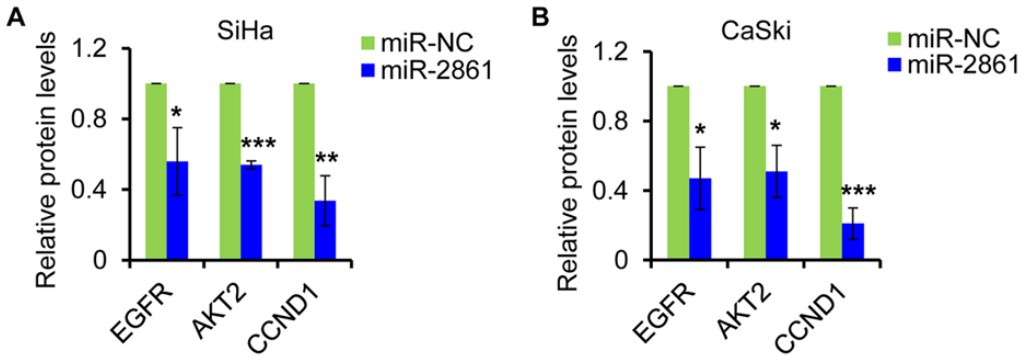

Figure S4. miR-2861 targets EGFR, AKT2, and CCND1 in cervical cancer cells. (A and B) Graph depicts the relative expression of EGFR, AKT2, and CCND1 protein levels in response to miR-2861 overexpression in cervical cancer SiHa (A) and CaSki (B) cells. Data are normalized to GAPDH levels and expressed as fold change from the control (miR-NC). Error bars indicate ± SD. * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

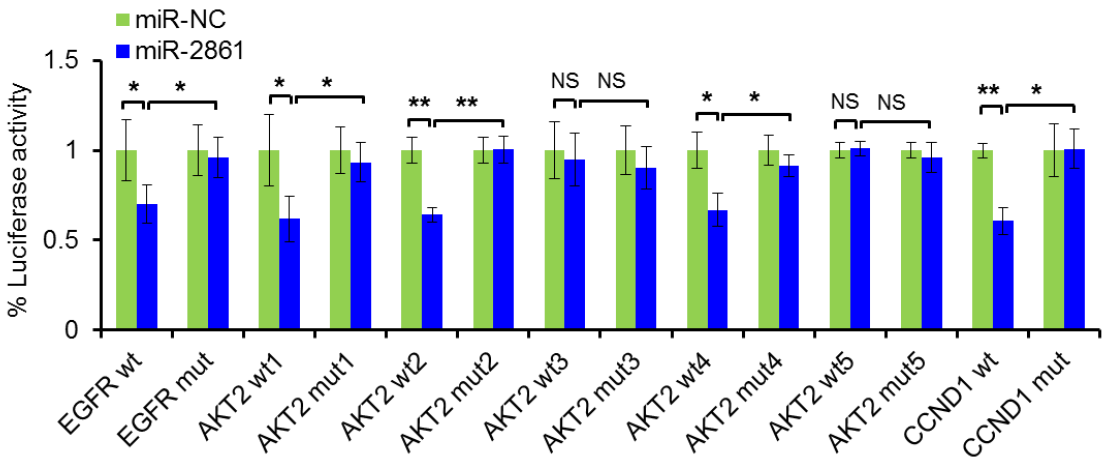

Figure S5. miR-2861 targets EGFR, AKT2, and CCND1 in HPV16 E6-expressing HaCaT (HaCaT-E6) cells. pmiR-GLO luciferase construct containing each wt (EGFR wt, AKT2 wt1, AKT2 wt2, AKT wt3, AKT wt4, AKT2 wt5, or CCND1 wt) or mutated (EGFR mut, AKT2 mut1, AKT2 mut2, AKT2 mut3, AKT2 mut4, AKT2 mut5, or CCND1 mut) 3'UTR was cotransfected with miR-2861 or miR-NC in HaCaT-E6 cells, respectively. The luciferase assay was then performed. Error bars, ± SD. * P<0.05; ** P<0.01; NS, not significant.

Table S1. Differentially expressed miRNAs between HPV16 E6 and vector control groups in HEK293T cells

Accession No.	Reporter Name	p-value	$\log_2$ (fold change)
MIMAT0004485	hsa-let-7e-3p	4.22E-03	4.94
MIMAT0022296	hsa-miR-5588-3p	6.63E-03	3.17
MIMAT0010367	hsa-miR-764	7.03E-03	5.70
MIMAT0003273	hsa-miR-605	7.16E-03	5.31
MIMAT0019808	hsa-miR-4707-3p	8.68E-03	3.63
MIMAT0003879	hsa-miR-758-3p	1.02E-02	3.11
MIMAT0019979	hsa-miR-4800-3p	1.04E-02	2.53
MIMAT0019722	hsa-miR-4655-3p	1.06E-02	3.16
MIMAT0004482	hsa-let-7b-3p	1.19E-02	4.03
MIMAT0019820	hsa-miR-4713-5p	1.45E-02	3.12
MIMAT0013802	hsa-miR-2861	1.47E-02	-0.81
MIMAT0010195	hsa-let-7a-2-3p	1.52E-02	3.83
MIMAT0022939	hsa-miR-939-3p	1.54E-02	3.44
MIMAT0002815	hsa-miR-432-3p	1.64E-02	3.25
MIMAT0002170	hsa-miR-412	1.80E-02	2.62
MIMAT0018065	hsa-miR-3646	1.82E-02	4.37
MIMAT0022726	hsa-miR-1306-5p	1.84E-02	1.80
MIMAT0009203	hsa-miR-449b-3p	1.88E-02	4.05
MIMAT0005921	hsa-miR-1267	1.89E-02	3.43
MIMAT0002173	hsa-miR-483-3p	1.98E-02	2.25
MIMAT0019940	hsa-miR-4436b-5p	1.98E-02	5.08
MIMAT0004986	hsa-miR-943	2.11E-02	3.52
MIMAT0016906	hsa-miR-4274	2.16E-02	2.17
MIMAT0004813	hsa-miR-411-3p	2.18E-02	4.20
MIMAT0022732	hsa-miR-3191-5p	2.18E-02	2.79
MIMAT0018085	hsa-miR-3663-3p	2.24E-02	2.67
MIMAT0016909	hsa-miR-4279	2.30E-02	2.21
MIMAT0019212	hsa-miR-3160-5p	2.33E-02	3.74

MIMAT0007884	hsa-miR-1910	2.36E-02	3.43
MIMAT0019957	hsa-miR-4787-3p	2.36E-02	2.91
MIMAT0025845	hsa-miR-6716-3p	2.47E-02	3.73
MIMAT0019789	hsa-miR-4695-3p	2.53E-02	4.14
MIMAT0022720	hsa-miR-1304-3p	2.59E-02	3.50
MIMAT0004703	hsa-miR-335-3p	2.63E-02	4.78
MIMAT0003300	hsa-miR-631	2.69E-02	2.96
MIMAT0004677	hsa-miR-34c-3p	2.90E-02	5.24
MIMAT0004607	hsa-miR-138-1-3p	3.03E-02	3.01
MIMAT0019219	hsa-miR-3619-3p	3.03E-02	3.01
MIMAT0019877	hsa-miR-3591-3p	3.32E-02	3.01
MIMAT0019226	hsa-miR-3150b-5p	3.34E-02	3.18
MIMAT0003888	hsa-miR-766-3p	3.35E-02	1.75
MIMAT0003298	hsa-miR-629-3p	3.42E-02	2.78
MIMAT0004615	hsa-miR-195-3p	3.49E-02	3.97
MIMAT0004946	hsa-miR-744-3p	3.55E-02	2.87
MIMAT0001639	hsa-miR-409-3p	3.60E-02	4.35
MIMAT0009451	hsa-miR-1976	3.62E-02	2.33
MIMAT0018350	hsa-miR-3935	3.93E-02	2.62
MIMAT0004804	hsa-miR-615-5p	4.00E-02	3.02
MIMAT0019854	hsa-miR-4731-3p	4.01E-02	2.97
MIMAT0000437	hsa-miR-145-5p	4.06E-02	2.51
MIMAT0000269	hsa-miR-212-3p	4.08E-02	3.53
MIMAT0004819	hsa-miR-671-3p	4.09E-02	3.22
MIMAT0005573	hsa-miR-1225-3p	4.10E-02	2.69
MIMAT0000450	hsa-miR-149-5p	4.26E-02	2.40
MIMAT0019857	hsa-miR-4733-5p	4.26E-02	3.31
MIMAT0021024	hsa-miR-5002-3p	4.34E-02	3.39
MIMAT0000227	hsa-miR-197-3p	4.62E-02	1.60
MIMAT0003310	hsa-miR-640	4.80E-02	2.85
MIMAT0019904	hsa-miR-4758-3p	4.88E-02	4.25