

Figure S1. Target sequence of hsa-miR126-3p into the psiCHECK-2 vector.

hsa-miR-126-3p Target sequence into the psiCHECKTM-2 Vector

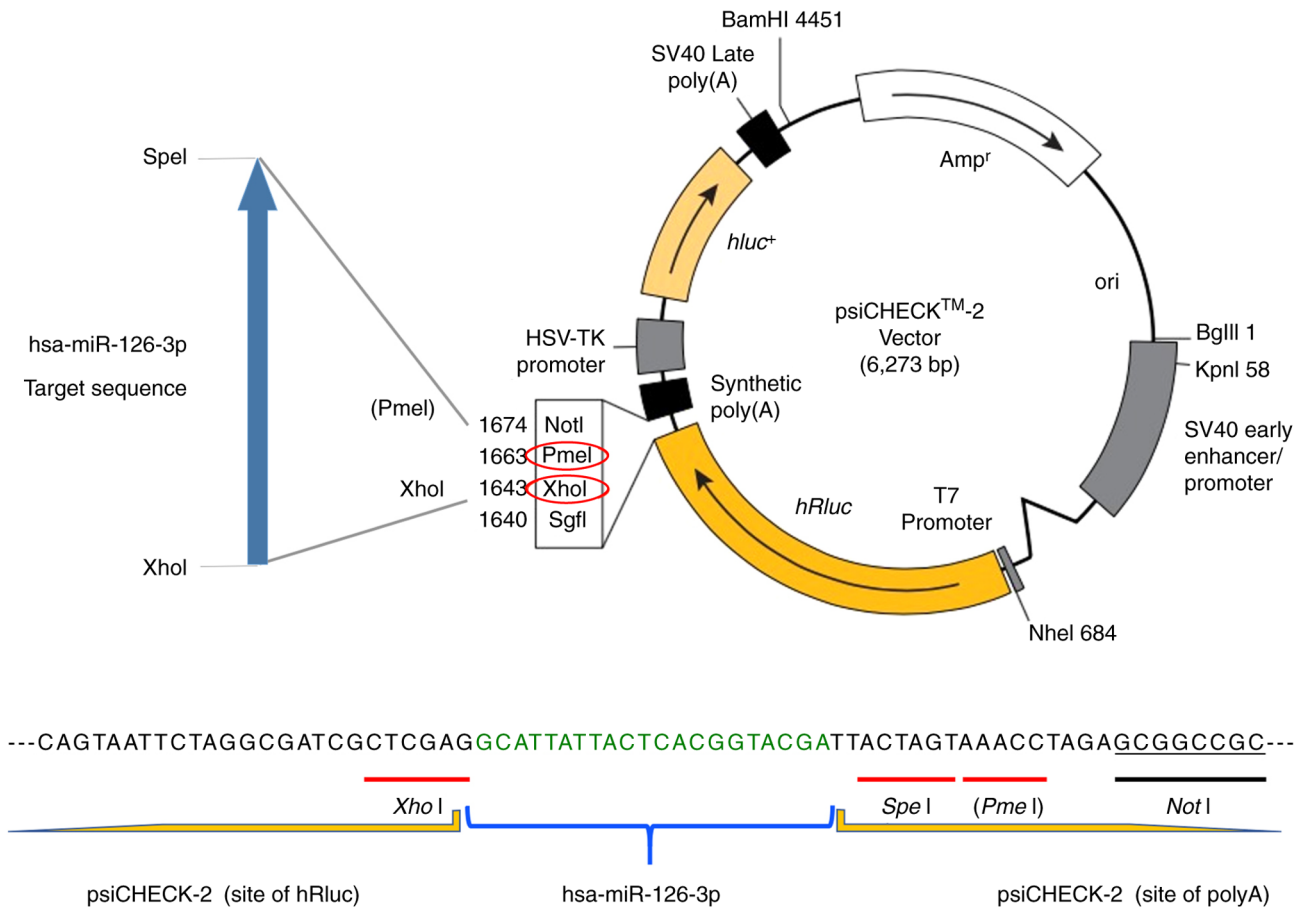


Figure S2. Relative *Renilla* luciferase activity is suppressed by transfection with miR-126-3p. The psiCHECK-2-miR126-3p and miR-126-3p mimic or negative control (NC) mimic were transfected into HeLa cells. The relative luciferase activity was normalized to control (Firefly) luciferase activity. Data were obtained from the mean of three independent experiments. Error bars represent standard deviations. Statistical analysis was performed by t-test. ***P<0.001.

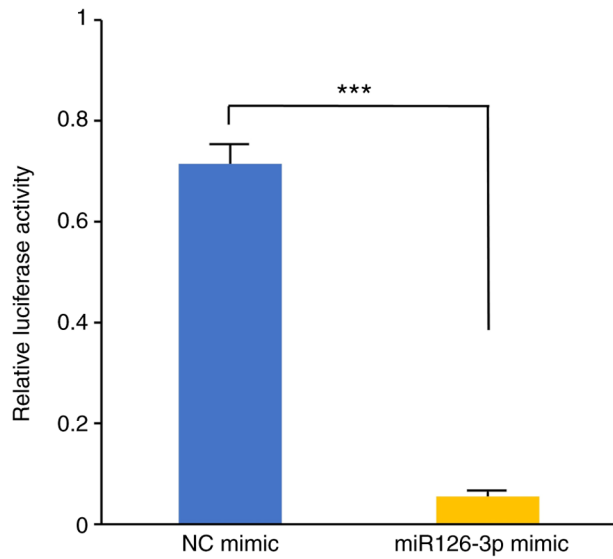


Figure S3. Relative amounts of miR-126-3p in cervical cancer cells. Eleven human cervical cancer cell lines (C33a, SKG-1, SKG-2, SKG-3a, SKG-3b, SiHa, HeLa, HT-3, ME-180, CaSki, and C-4I) were obtained from Keio University, Japan (C33a, SKG-1, SKG-2, SKG-3a, SKG-3b, SiHa and HeLa), or the American Type Culture Collection (HT-3, ME-180, CaSki and C-4I). The identities of the analyzed cell lines obtained from Keio University were confirmed by short terminal repeat genotyping, which revealed a correspondence of >80% of the markers tested. The cell lines were cultured in Ham's F-12 (Wako; C33a, SKG-1, SKG-2, SKG-3a, SKG-3b, SiHa, HeLa), RPMI-1640 (Gibco; Thermo Fisher Scientific, Inc.; CaSki), McCoy's 5a (Gibco; Thermo Fisher Scientific, Inc.; HT-3 and ME-180), or Waymouth MB 752/1 (Gibco; Thermo Fisher Scientific, Inc.; C4I), supplemented with 10% fetal bovine serum (Gibco; Thermo Fisher Scientific, Inc.), 10 μ g/ml penicillin, and 10 μ g/ml streptomycin (Wako) and maintained at 37°C in 5% CO₂. Cervical cancer cells were harvested and TRIzol reagent (Thermo Fisher Scientific, Inc.) was used to isolate total RNA including miRNAs according to the manufacturer's instructions. The isolated RNA was dissolved in 100-200 μ l RNase-free water depending on the volume of precipitation. RNA concentration and purity were determined by optical density measurement using NanoVue (GE Healthcare UK Ltd.). MicroRNAs were quantified using TaqMan[®] MicroRNA Assays (Thermo Fisher Scientific, Inc.) with modifications. Briefly, 5.34 ng of total RNA was reverse transcribed (RT) by a TaqMan[®] MicroRNA RT Kit. The total volume of 8 μ l RT reactions contained 10X RT buffer, 0.08 μ l of 100 mM dNTPs with dTTP, 0.1 μ l of RNase-inhibitor (20 U/ μ l), 0.53 μ l of MultiScribe[™] Reverse Transcriptase (50 U/ μ l), 1.6 μ l of each of the microRNA specific stem-loop primers (hsa-miR-126, 002228 and RNU48 as a Mature miRNA Control, 001006; Thermo Fisher Scientific, Inc.) and 2.67 μ l (5.34 ng as the template) of input RNA. The mixture was incubated at 16°C for 30 min, 42°C for 30 min, and 85°C for 5 min. Subsequently, quantitative real time-PCR was performed using a 7900 Real-Time PCR system (Thermo Fisher Scientific, Inc.). For each 20 μ l PCR reaction, 20X TaqMan[®] MicroRNA Assays containing PCR primers and probes (5'-FAM and 3'-TAMRA), 1.5 μ l of RT product, and 10 μ l of 2X TaqMan[®] Gene Expression Master Mix (Thermo Fisher Scientific, Inc.) were mixed together. The reaction was first incubated at 50°C for 2 min and 95°C for 10 min, followed by 50 cycles of 95°C for 15 sec and 60°C for 1 min. Data were analyzed with RQ Manager 1.2 (Thermo Fisher Scientific, Inc.) with the automatic Ct setting for adapting baselines and thresholds for Ct determination. Relative fold changes were determined from the Ct values with the 2^{- $\Delta\Delta$ Ct} method. Data were normalized to RNU48 to account for possible differences in the amount of starting RNA. x-axis represents cervical cells; y-axis represents relative expression level compared with cells. SiHa, SKG-3a, SKG-3b and CaSki contained HPV16 genome; SKG-1, SKG-2, HeLa and C4I contained HPV18 genome; ME-180 contained HPV68 genome; C33a and HT-3 were HPV-negative.

