Supplementary Information

Star-PAP controls HPV E6 regulation of p53 and sensitizes cells to VP-16

Weimin Li[1,2] and Richard Anderson[1,2*]

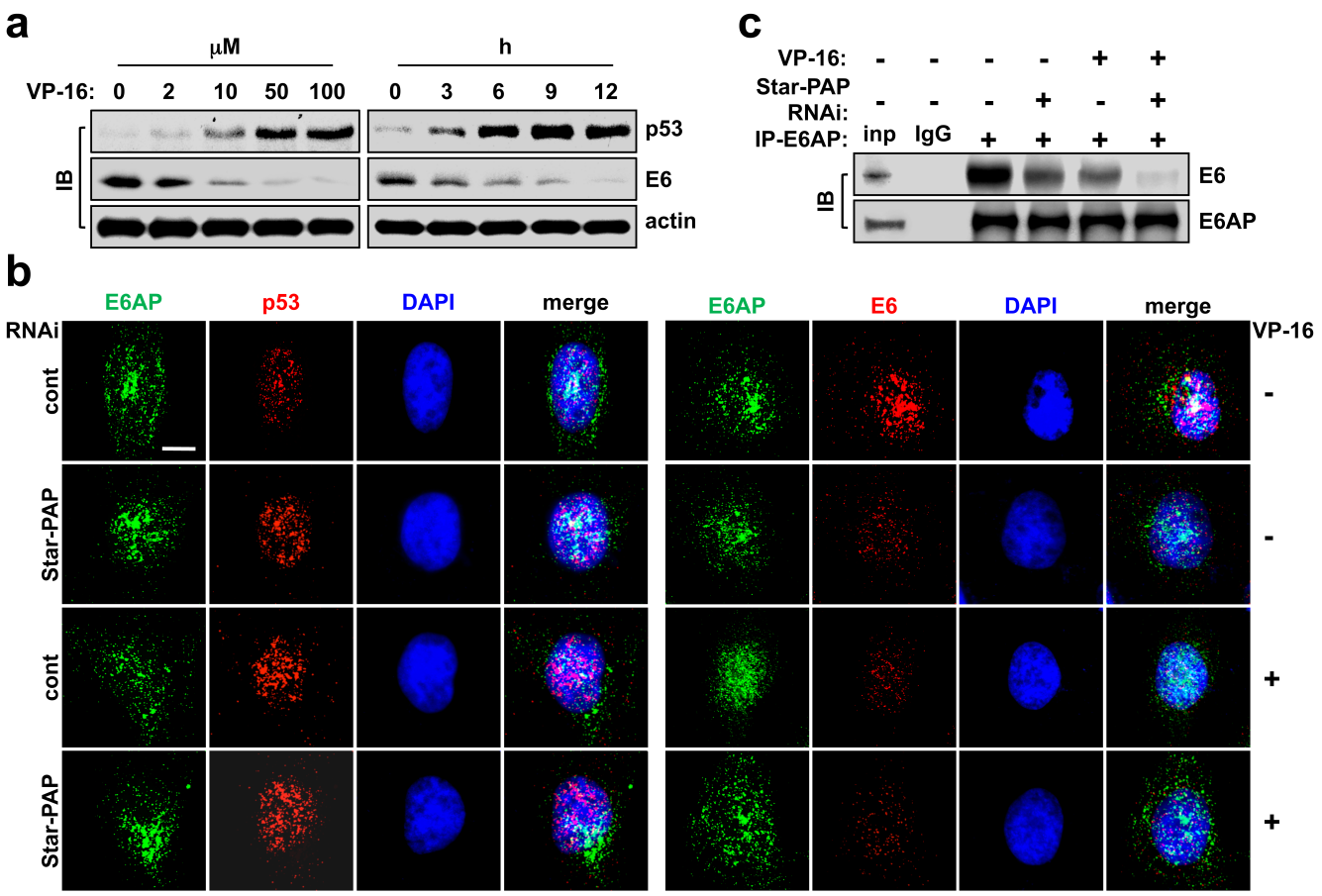
[1]Molecular and Cellular Pharmacology Program, [2]University of Wisconsin-Madison, School of Medicine and Public Health, Madison, Wisconsin 53706, USA

1. **Supplementary Figure1:** Star-PAP regulates basal and VP-16-induced nuclear accumulation of p53 by interfering with the E6/E6AP proteolytic targeting complex for p53 degradation.

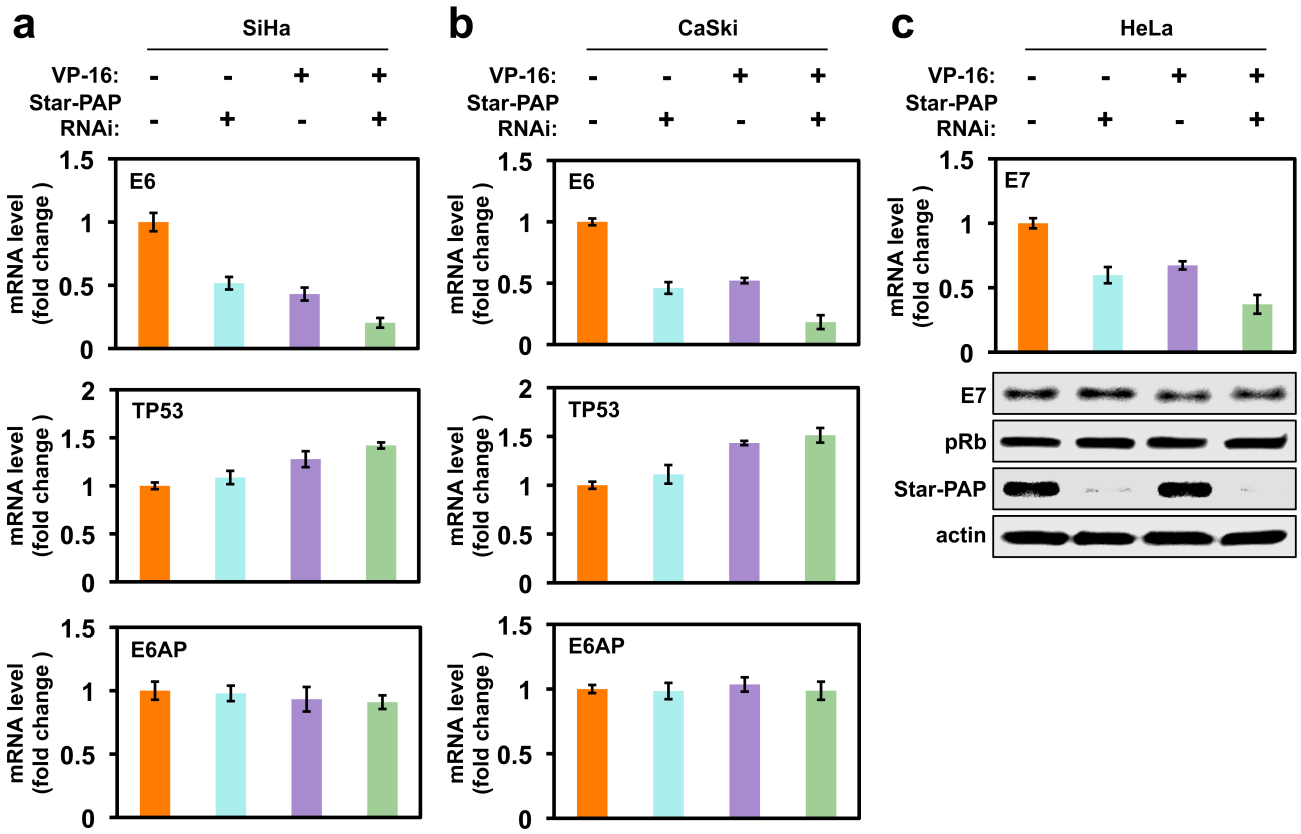
2. **Supplementary Figure 2:** Star-PAP and DNA damage signaling mediate E6 and E7 mRNA levels in different high-risk HPV-positive cells.

3. **Supplementary Figure 3:** Star-PAP regulation of p53 expression is not through Mdm2-mediated degradation in HeLa cells.

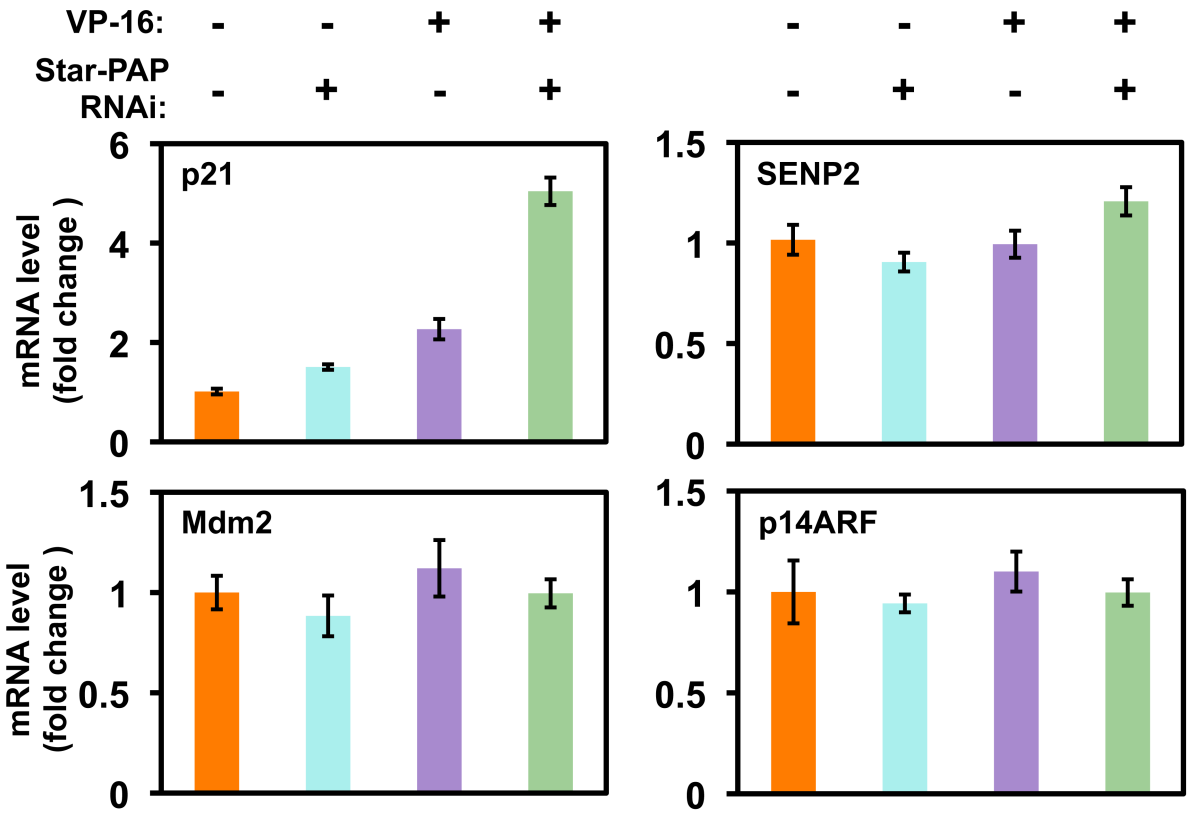
4. **Supplementary Figure 4:** Star-PAP mediates DNA-damage-regulated cell cycle progression.



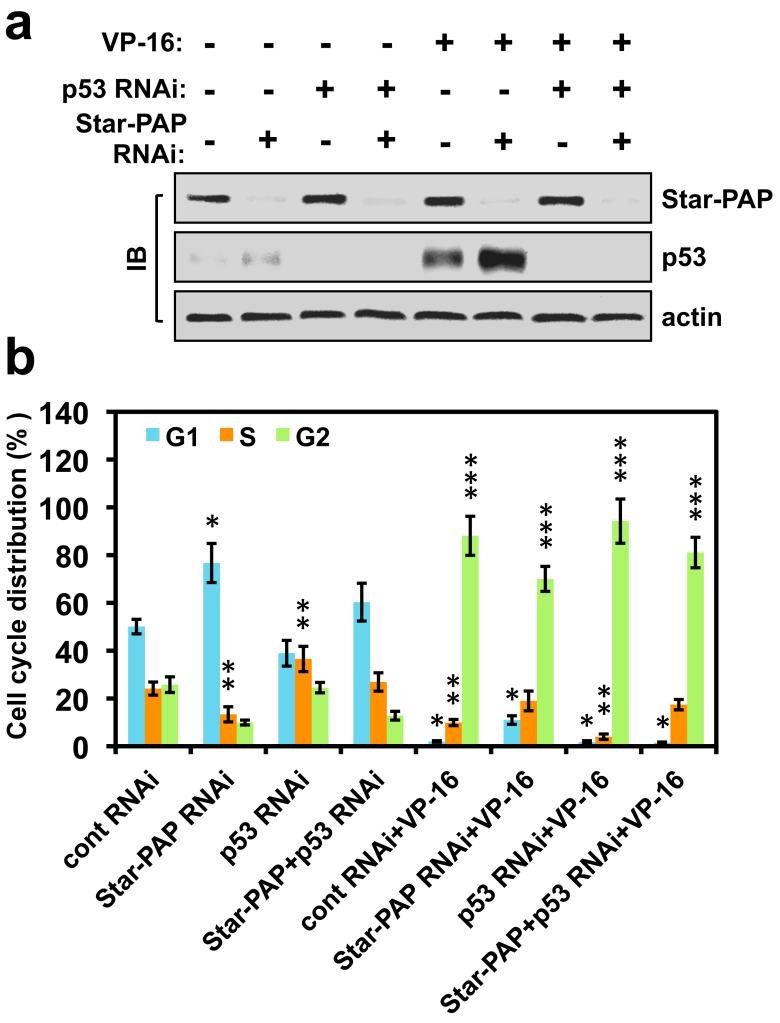
Supplementary Figure 1 Star-PAP regulates basal and VP-16-induced nuclear accumulation of p53 by interfering with the E6/E6AP proteolytic targeting complex for p53 degradation. (a) VP-16 dose (for 6 h) and time (at 50 μM) dependently induces p53 and diminishes E6 expression in HeLa cells as shown by IB. (b) IF staining demonstrated increased accumulation of p53 and decreased E6 level in HeLa cell nucleus as well as attenuated association of p53, E6 and E6AP after Star-PAP knockdown or/and VP-16 treatment (50 μM, 6h). Scale bar = 10 μm. (c) E6 and E6AP interaction was decreased upon down-regulation of Star-PAP and with or without administration of VP-16 (50 μM, 6h) as examined by immunoprecipitation (IP) and IB.



Supplementary Figure 2 Star-PAP and DNA damage signaling mediate E6 and E7 mRNA levels in different high-risk HPV-positive cells. The effect of Star-PAP knockdown and VP-16 treatment (50 μM, 6h) on E6, TP53 and E6AP mRNA expression in HPV-16-positive SiHa (a) and CaSki (b) cells was analyzed by qRT-PCR. (c) E7 mRNA expression in HeLa cells under the same experimental conditions was examined as described above, and the protein levels of E7 as well as its target protein pRb expression were also evaluated. The mRNA levels were normalized to GAPDH expression and to mock-treated control. Error bars represent standard error of the mean of three independent experiments with triplicates for each experimental condition. Antibodies used: Mouse monoclonal anti-HPV-18 E7 (Santa Cruz Biotechnology, #sc-365035); Mouse monoclonal anti-pRb (Santa Cruz Biotechnology, #sc-102).



Supplementary Figure 3 Star-PAP regulation of p53 expression is not through Mdm2-mediated degradation in HeLa cells. Quantitative Real-time RT-PCR was performed to check target gene expression in HeLa cells treated with or without VP-16 (50 μM, 6h) in the presence or absence of Star-PAP knockdown. p21 mRNA expression was used as an indicator of p53 transactivation potential, and cellular Mdm2, SENP2 and p14ARF mRNA levels were also examined. Target mRNA abundance was normalized to GAPDH expression as described formerly, and the mRNA expression levels displayed on charts were normalized to mock-treated control. Error bars represent standard error of the mean of three independent experiments with triplicates for each experimental condition. Primers used for the analysis: p21 forward 5'-gacaccactggagggtgact-3', reverse 5'-caggtccacatggtcttcct-3'; MDM2 forward 5'-ggtgggagtgatcaaaagga-3', reverse 5'-acacagagccaggctttcat-3'; SENP2 forward 5'-aggtacattggagcctggtg-3', reverse 5'-agaggcatctggtgctgagt-3'; P14ARF forward 5'-agggttttcgtggttcacat-3', reverse 5'-ctgcccatcatcatgacct-3'.



Supplementary Figure 4 Star-PAP mediates DNA damage- and p53-regulated cell cycle progression. (a) The knockdown of Star-PAP and p53 in HeLa cells used for FACS, cell proliferation and colony formation assays were assessed by IB. VP-16 treatment at the concentration of 50 μM (6h) was used for cells subjected to FACS, and different dose and time of VP-16 treatment were used for proliferation and colony formation assays as indicated. (b) FACS analysis of cell cycle status in the presence or absence of Star-PAP or/and p53 knockdown and VP-16 treatment. The percentage of cell numbers in individual cell phases under the indicated conditions was indicated. Propidium Iodide (PI) staining and cell cycle analysis were carried out as described[34]. Cells were collected by trypsinization, washed, fixed with 70% ethanol and stained with PI followed by analysis using a FACSCalibur flow cytometer (UW-Madison). Error bars represent standard deviation of three independent experiments. For statistics, individual experimental conditions are compared to control group. * = $P \leq 0.05$ for G1 phase; ** = $P \leq 0.05$ for S phase; *** = $P \leq 0.05$ for G2 phase (t-test).