SUPPLEMENT

Methods

Transient HPV18 ori cDNA Replication assay

pSGE1 and pSGE2 plasmid expressing HPV 18 E1 and E2, pOri177, containing the HPV 18 origin (nt 7800-7857/1-119), were described before (Sverdrup & Khan, 1994). Transient replication assay was performed as described before (Sverdrup & Khan, 1994). C33A cells were co-transfected with 0.5 µg of pOri177 and 3 µg of pSGE1 and pSGE2 plasmid using calcium phosphate transfection protocol. Where indicated, 4 µg of siP56, siControl or siTLR5 were also co-transfected. 14 hrs post-transfection, cells were treated with IFN-β. After 3 days, low molecular weight DNA was extracted (Hirt, 1967). Half of the DNA was digested by Dpn-I and linearize by EcoRI; the other half was digested with EcoRI. The DNA was analyzed by southern bloting with 32-P labeled pUC19 probe generated by using a random-primer labeling kit (Amersham). The blots were visualized by autoradiography and replicated DNA was quantitated using PhosphoImager (molecular Dynamics) and values normalized to input of E1 and E2.

Preparation of mammalian cell extracts and cell free HPV DNA replication

Cellular extracts from 293 cells were prepared according to Li and Kelly (Li & Kelly, 1984). The E1 and E1/E2 cell-free DNA replication was carried out as described with minor modifications (Kuo et al, 1994). Briefly, a 25 µl reaction mixture contained purified E1 protein, E2 protein, DNA template, and 293 cell extract (10 mg/ml) and 20 mM HEPES buffer, pH7.5, 200 µM each UTP, GTP, CTP and 4 mM ATP, 100 µM each dATP, dGTP, dTTP, 25 µM dCTP, 40 mM phosphocreatine and 100 µg/ml creatine phosphokinase; 2.5 µCi of [α32-P] dCTP was added after 1hrs. Replication was
terminated by adding 200 µl of 20 mM Tris-Cl pH7.5, 10 mM EDTA, 0.1% SDS, and 20 µg/ml RNaseA, followed by incubation at 37°C for 15 min. Proteinase K was then added to 200 µg/ml and incubation continued for another 30 min at 37°C. The reaction mixture was extracted with phenol and chloroform: isoamyl alcohol precipitated with 2.5M ammonium acetate, 70% ethanol wash, and analyzed by 0.8% agarose gel electrophoresis. Dried gels were exposed to X-ray film and quantitated using phosphoImager (Molecular Dynamics).

**Immunofluorescence**

P2.1 cells were grown on cover slips in 6 well tissue culture plates and transfected using FuGENE6 with 4 µg of HPV 11 pEGFPE1 and cotransfected with 4 µg of vector alone, pCDNA3-P56, or myc-P54 , or stimulated with human IFN-β (1000 units/ml, Calbiochem). After 18 hrs cells were fixed with 4% paraformaldehyde for 30 min and permialized with 0.2% Triton X-100 for 15 min. The cells were then blocked with PBS containing 0.02% Tween-20, 3% bovine serum albumin and 3% goat serum at 4°C overnight and incubated with anti-P56 or anti-myc (1:2000) antibody for 2 hrs at room temperature. Cells were washed and further incubated with anti-rabbit-Alex Flour 594 (1:1,500; Molecular Probes) for 1 hrs at room temperature. After incubation, cells were washed and covered with Vectashield containing 4’, 6’-diamino-2-phenylindole (DAPI, Vector Labs). Thereafter the slides were imaged on a Leica digital fluorescence microscope. Autofluorescence of GFP-E1 was examined directly. For P56 nuclear localization immunofluorescence, HT1080 cells were transfected with 4µg of NLS P56 construct and stained as described above with anti-Flag antibody (1:2000) and anti-mouse Alex Flour 488 (1: 2000; Molecular Probes).
Expression and purification of full length E1 and E2 protein from recombinant baculovirus expression system

HPV11 E1 and E2 proteins were expressed in insect cells using the BAC-to-BAC baculovirus expression system (Invitrogen). Sf21 cells were infected at a 10 multiplicity of infection (MOI) for 60 hrs with baculovirus containing recombinant bacmid DNA encoding E1 or E2 proteins, extracted in Buffer A [20 mM Tris-HCl pH 8.0, 1.5 mM MgCl2, 10% glycerol, 1 mM DTT and complete protease inhibitor cocktail (Roche)] by Dounce homogenization (20 strokes) and subjected to centrifugation at 13,000 rpm for 15 min. The E1 and E2 proteins, containing His-tag at their N-terminus were purified by affinity chromatography using Ni-NTA agarose beads and concentrated the protein using Amicon ultrafiltration unit.

Purification of recombinant protein from E.Coli

E. Coli BL21(DE3) pLys (Novagen) were transformed with pET15b/P56 or M2P56 and expression of His-tagged P56 or M2P56 was induced by 1mM isopropyl β-D-thiogalactopyranoside (IPTG) for 12 hrs at 30°C. Protein purification was done using Ni-NTA superflow beads (Qiagen) as described before (Terenzi et al, 2005).
Figure legends

Figure S1:

Co-immunoprecipitation of HPV11E1 and P56. HT1080 cells were transfected with plasmids expressing GFP fused HPV 11 E1 and cells were either co-transfected with P56 expressing plasmid (left panel) or treated with IFN-β to induce the expression of P56 (right panel). The cell extracts were immunoblotted (IB) directly or after immunoprecipitation (IP) using indicated antibodies.

Figure S2:

M2P56 does not interact with HPV11E1. HT1080 cells were co-transfected with plasmid expressing GFP fused HPV 11 E1 and either flag tagged P56 or point mutants of TPR 2 (M2P56). The cell extracts were immunoblotted (IB) directly or after immunoprecipitation (IP) using indicated antibodies.

Figure S3:

P56 does not inhibit E1 synthesis. C33A cells were either mock infected or infected with lentivirus expressing P56 (LV56) and 12 hrs post-infection cells were transfected with myc-E1 expressing plasmid. Expression levels of E1 in presence of P56 protein were detected by immunoblotting (IB) with Myc antibody.

Figure S4:

NLSP56 interacts with E1. C33A cells were infected with lentivirus expressing Wt or NLS P56 protein and transfected with myc-E1. Cell extracts were immunoblotted (IB) directly or after immunoprecipitation (IP) using indicated antibodies.
Figure S5:

**Cellular replication assay in presence of neutralizing antibodies to IFN.** The plasmid pOri177 (which contains the HPV18 origin) and expression vectors of E1 and E2 were co-transfected into C-33A cells and then cells were treated with IFN-α alone or in presence or absence of IFN-α antibody. Replicated ori DNA was quantitated and normalized. Data represent as means of 3 independent experiments.

Table S1:

**Effects of IFN on cell cycle.** C33A cells were treated with increasing doses of IFN-β or staurosporine for 16 hrs. Quantitations of cell populations at various stages of cell cycle were measured by flow cytometry.
Supplemental Figure 1

(gfp-E1 + P56) + (kDa) (gfp-E1 + IFN-β) + (kDa)

IP α-P56
IB α-gfp
IB α-gfp
IB α-P56

115
115
64
64
Supplemental Figure 2

gfp-E1  -  +  +  +
Flag-M2P56 -  +  -  -
Flag-P56  +  -  +  -

(kDa)

IP α-Flag
IB α-gfp
IB α-gfp
IP α-Flag

115 115 -64
Supplemental Figure 3

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<th>Myc-E1</th>
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IB α–myc
- 180
- 115
- 62

IB α–P56
- 64
### Supplementary Figure 4

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<th>Condition</th>
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<th>P56(LV)</th>
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**Western Blot Analysis**

- **IP α-P56**: +
- **IB α-myc**: +
- **IB α-P56**: +
- **IB α-myc**: +

- Protein bands at 115 kDa and 64 kDa.
Supplemental Figure 5

% Replication

−  Ab  Ab+ IFN

IFN
### Table 1

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