

SUPPLEMENTARY INFORMATION

MATERIALS AND METHODS

Plasmids.

The HPV16 plasmid pC97ELsL has been described previously [8, 9]. Construction of **phnRNP F**, **phnRNP I**, **phnRNP A2** and **phnRNP Q** have been described previously [13] and so has **phnRNP A1** [15] and **phnRNP C1** [4]. Flag-tagged **hnRNP D**-plasmids were generously provided by Dr. R.J. Schneider [12], **hnRNP G** plasmid by Dr. I. C. Eperon [11], histidine and myc-tagged **hnRNP L** plasmid by Dr. S. Guang [5] and histidine and myc-tagged **hnRNP R** plasmid by Dr. P. Xu [6]. **phnRNP AB** encoding myc-tagged **hnRNP AB** transcript variant 1 (RC204360), **phnRNP DL** encoding **hnRNP DL** transcript variant 2 (RC204064) and **pRALYL** encoding myc-tagged **RALYL** (RC206313) were purchased from OriGene Technologies Inc. **phnRNP E1** contains the **hnRNP E1** (PCBP-1) open reading frame with a FLAG-tag in the C-terminus driven by a CMV promoter. It was constructed by PCR-amplification of the **hnRNP E1** coding sequence from pGEX-2TK-PCBP-1 (generously provided by Dr. H. Leffers) [7] with primers ES (5'-GGCGCGCAAGAAatggatgccggtgtgactgaaag-3') and EA (5'-CCTCGAGctaCTTATCGTCGTCATCCTTGTAATCgctgcaccccatgccc-3') followed by digestion with PteI and XhoI and subcloning into pCL086 [3]. **phnRNP K** contains the **hnRNP K** open reading frame with a FLAG-tag in the C-terminus driven by a CMV promoter. It was constructed by PCR-amplification of the **hnRNP K** coding sequence from pGEX-KT-**hnRNP K** (generously provided by Dr. K. Bomsztyk) [1] with primers KS (5'-GGCGCGCAAGAAatggatgccggtgtgactgaaag-3') and KA (5'-CCTCGAGctaCTTATCGTCGTCATCCTTGTAATCgctgcaccccatgccc-3') followed by digestion with PteI and XhoI and subcloning into pCL086 [3]. **phnRNP F** and **phnRNP H** contain the **hnRNP F** or **hnRNP H** open reading frames driven by a CMV promoter. They were constructed by PCR-amplification of either the **hnRNP F** or **hnRNP H** coding sequence from pET-15B-**hnRNP F** or pET-15B-**hnRNP H** (generously provided by Dr. D. Black) [2] followed by subcloning into pCL086 [3]. Plasmids expressing SR proteins have been described previously [14].

Cells

HeLa cells were cultured in Dulbecco's modified Eagle medium (GE Healthcare Life Science Hyclone Laboratories) with 10% bovine calf serum (GE Healthcare Life Science Hyclone Laboratories) and 1% penicillin-streptomycin (Gibco Thermo Fisher Science).

Transfections

Transfections of HeLa cells were carried out using Turbofect according to the manufacturer's instructions (Thermo Fisher Science). Turbofect was mixed with plasmid DNA and incubated

at room temperature for 15 min prior to drop-wise addition to 60-mm plates with subconfluent HeLa cells. Cells were harvested at 20hrs post transfection. Each plasmid was transfected in a minimum of three independent experiments.

RNA extraction and RT-PCR

Total RNA was extracted using TRI Reagent and Direct-zol RNA MiniPrep kit (ZYMO Research) according to the manufacturer's protocol. 1µg of total RNA were reverse transcribed in a 20 µl reaction at 37°C by using M-MLV Reverse Transcriptase (Invitrogen) and random primers (Invitrogen) according to the protocol of the manufacturer. One microliter of cDNA was subjected to PCR amplification. HPV16 late L1 mRNAs were amplified with RT-PCR primers 97S or 773S and L1A (**Supplementary Table T1**). HPV16 mRNAs spliced from HPV16 5'-splice site SD226 to 3'-splice sites SA409, SA526, and SA742 were PCR amplified with RT-PCR primers 97S and 880A (**Supplementary Table T1**). HPV16 mRNAs spliced from 5'-splice site SD880 to 3'-splice sites SA2709 or SA3358 were amplified with RT-PCR primers 773S and E2A or E4A (**Supplementary Table T1**). GAPDH cDNA was amplified with primers gapdhf and gapdhr (**Supplementary Table T1**).

Secreted luciferase assay

The *Metridia longa* secreted luciferase activity [10] in the culture medium of the C33A2 cells was monitored with the help of the "Ready To Glow" secreted luciferase reporter assay according to the instructions of the manufacturer (Clontech Laboratories). Briefly, 50 ul of cell culture media were mixed with 5 ul of secreted luciferase substrate in reaction buffer, and the luminescence was monitored in a Tristar LB941 luminometer (Berthold Technologies).

Quantitations.

The software used to determine band intensity in RT-PCR gels is "Image Lab 6.0.1" and quantitations were performed with the software "Prism GraphPad 8.4.0".

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Supplementary Table T1. RT-PCR primers.

Primer name	Sequence (5' - 3')
97S	GTCGACCTGCAATGTTTCAGGACCC
773S	GCACACACGTAGACATTCGTACTTTG
880A	GATCAGCCATGGTAGATTATGGTTTC
E2A	CCTGACCACCCGCATGAACTTCC
E4A	TGCTGCCTAATAGTTTCAGGAGAGG
L1A	GCAACATATTCATCCGTGCTTACAACC
FSet3	GCACCCCCTTTAACAGTAGATCC
RSet3	TACAGATGGGTCAGTGAAAGTG
gapdhf	ACCCAGAAGACTGTGGATGG
gapdhr	TTCTAGACGGCAGGTCAGGT

SUPPLEMENTARY FIGURE LEGEND.

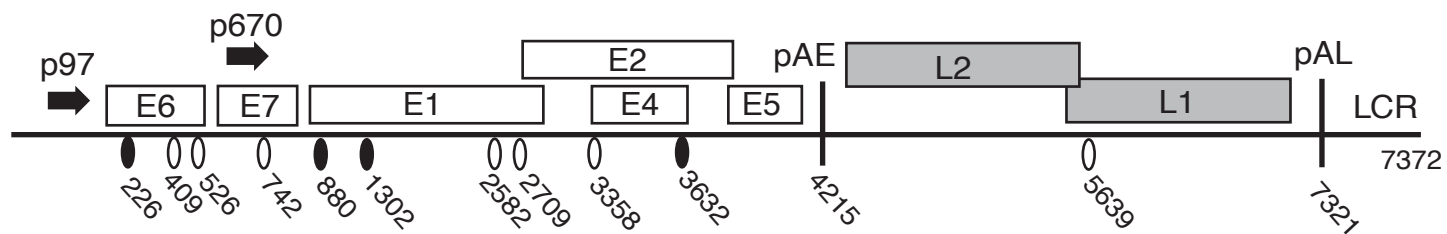
SUPPLEMENTARY FIGURE S1. (A) Schematic representation of the HPV16 genome and **(B)** the HPV16 subgenomic pC97ELsL reporter plasmid. Transcription of the HPV16 sequences in the pC97ELsL plasmid is driven by the human cytomegalovirus immediate early promoter (CMV). HPV16 splice sites are indicated. Numbers refer to the HPV16 reference strain HPV16R ([GeneBank: K02718.1](#)). Early and late polyadenylation signals pAE and pAL are indicated. p97 and p670 indicate HPV16 early and late promoters, respectively. IRES, poliovirus 2A internal ribosome entry site; sluc, secreted luciferase. **(C)** Alternatively spliced HPV16 L1 mRNAs produced by subgenomic HPV16 expression plasmid pC97ELsL are indicated. Arrows indicate positions of RT-PCR primers. **(D, E)** RT-PCR on RNA extracted from HeLa cells transfected with pC97ELsL in the absence (-) or presence of the indicated hnRNP D expression plasmids. RT-PCR was performed with primers FSet3 and RSet3 in the presence **(D)** or absence of RT **(E)**.

SUPPLEMENTARY FIGURE S2. Secreted luciferase (sLuc) activity in the cell culture medium of HeLa cells transfected with pC97ELsL in the absence (-) or presence of 2-fold serially diluted **phnRNP Q** (2.0ug, 1.0ug, 0.5ug and 0.25ug) **(A)** or 2-fold serially diluted **phnRNP R** (2.0ug, 1.0ug, 0.5ug and 0.25ug) **(B)**. **RT-PCR on RNA extracted from HeLa cells transfected with pC97ELsL in the absence (-) or presence of 2-fold serially diluted phnRNP Q (2.0ug, 1.0ug, 0.5ug and 0.25ug) (C) or 2-fold serially diluted phnRNP R (2.0ug, 1.0ug, 0.5ug and 0.25ug) (D)**. RT-PCR was performed with RT-PCR primers 773S and L1A. The HPV16 splice sites used to generate the mRNAs amplified by the RT-PCR primers are indicated to the right. **Gapdh** cDNA was PCR-amplified by primers gapdhf and gapdhr. M, molecular size marker. **(E) Quantitation of RT-PCR bands in (C). (F) Quantitation of RT-PCR bands in (D).**

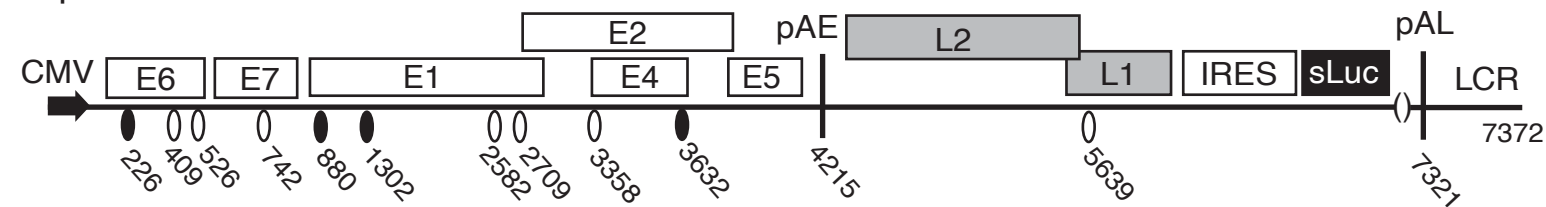
SUPPLEMENTARY FIGURE S3. (A) Schematic representation of the HPV16 subgenomic pC97ELsL reporter plasmid. Transcription of the HPV16 sequences in the pC97ELsL plasmid is driven by the human cytomegalovirus immediate early promoter (CMV). HPV16 splice sites are indicated. Numbers refer to the HPV16 reference strain HPV16R. Early and late polyadenylation signals pAE and pAL are indicated. IRES, poliovirus 2A internal ribosome entry site; sLuc, secreted luciferase. **(B, C)** Schematic representation of HPV16 early mRNAs produced by pC97ELsL. Numbers represent HPV16 splice sites. RT-PCR primers 97S, 773S, 880A, E2A and E4A are indicated.

SUPPLEMENTARY FIGURE S4. RT-PCR on RNA extracted from HeLa cells transfected with pC97ELsL in the absence (-) or presence of 2-fold serially diluted **phnRNP Q** (2.0ug, 1.0ug, 0.5ug and 0.25ug) **(A-E)** or 2-fold serially diluted **phnRNP R** (2.0ug, 1.0ug, 0.5ug and 0.25ug) **(G-J)**. RT-PCR was performed with the indicated primer pairs. The HPV16 splice sites used to generate the mRNAs amplified by the RT-PCR primers are indicated to the right. **Gapdh** cDNA was PCR-amplified by primers gapdhf and gapdhr. M, molecular size marker.

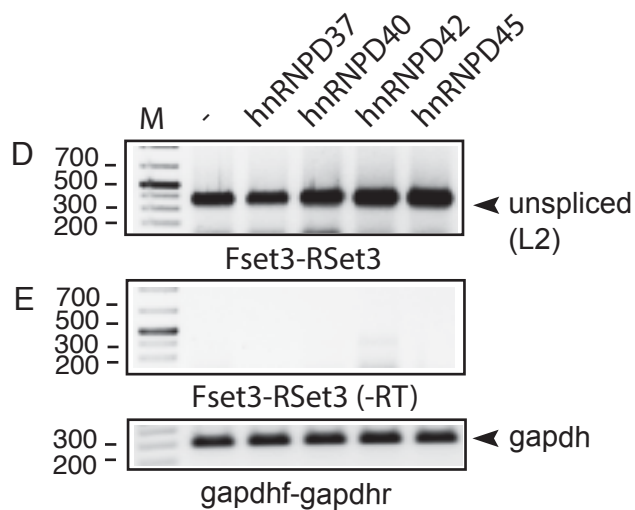
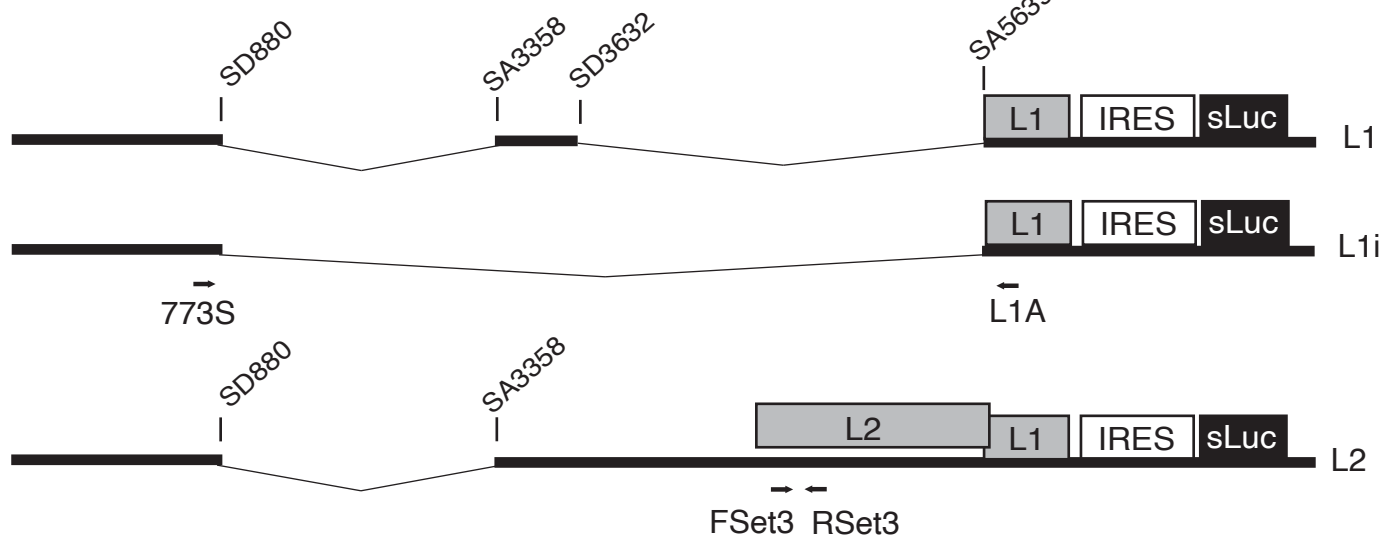
A HPV-16 genome

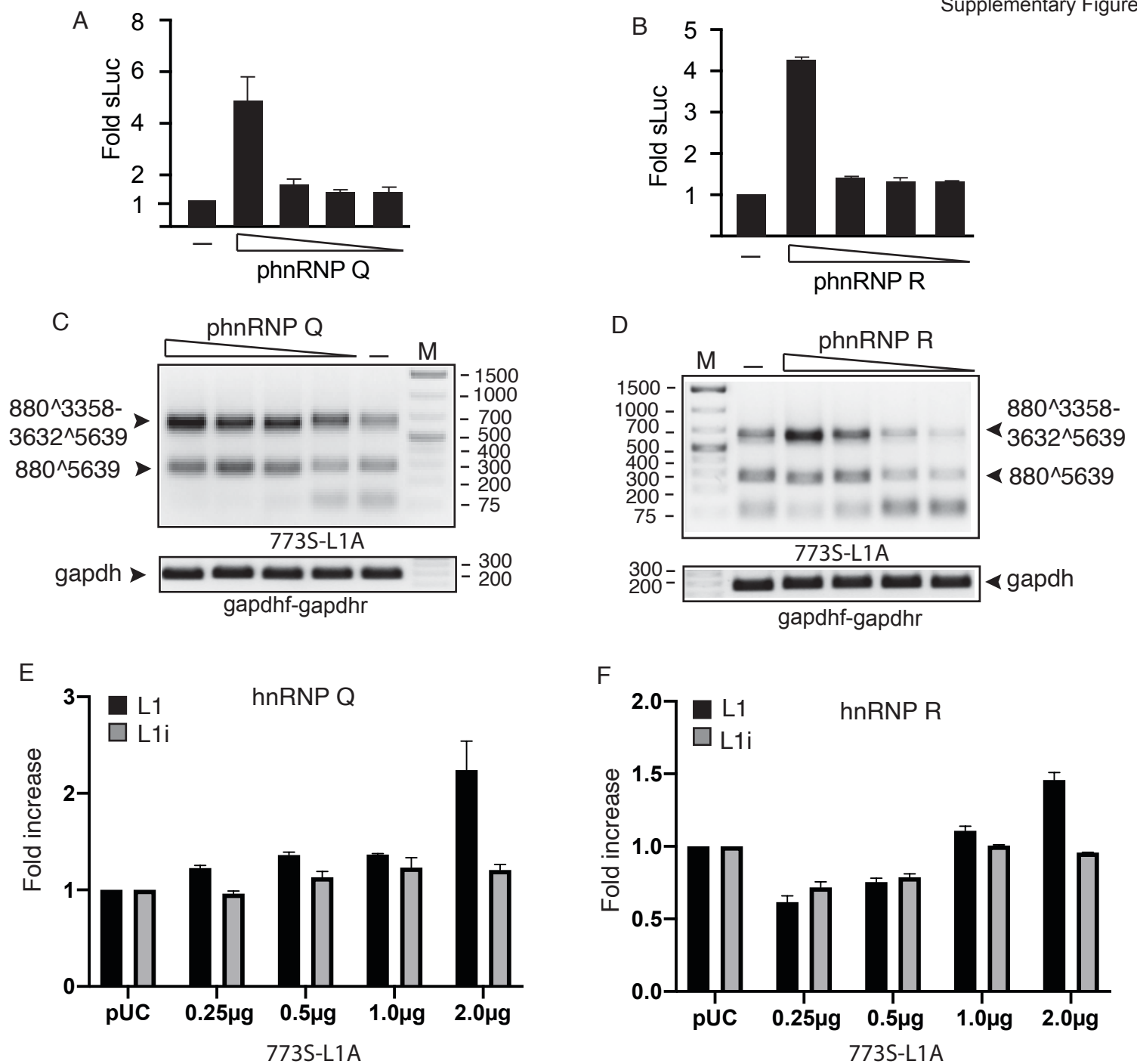


B pC97ELsL

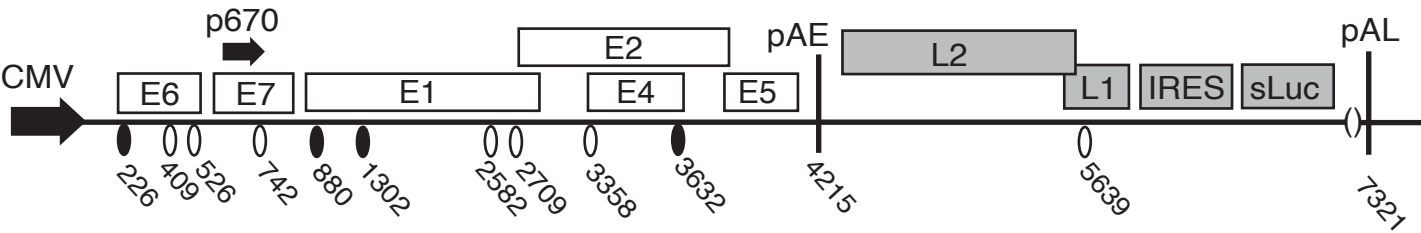


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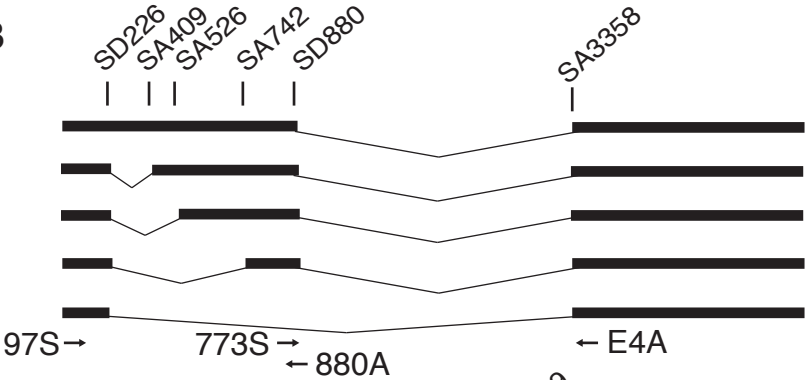




A pC97ELsL



B



C

