EXTENDED EXPERIMENTAL PROCEDURES

Cell Cultures

Primary keratinocytes were cultured from small biopsy specimens of healthy adult individuals. The cells were maintained in keratinocyte Growth Medium (KGM): 75% DMEM, 25% Nutrient mixture F-12 (HAM), 10% fetal bovine serum, 5 μg/ml insulin, 0.4 μg/ml hydrocortisone, 10^{-10} M cholera toxin, 10 ng/ml epidermal growth factor, 1.8×10^{-4} M adenine, 5 μg/ml transferrin, 2×10^{-9} M T3, 100,000 U/l penicillin, 100 μg/l streptomycin, 0.1 mg/ml amphotericin. The effect of the empty LXSN vector was tested in only two of the donors since primary keratinocytes from adult donors have a very limited number of proliferations (unless they are expressing E6/E7) and the number of cells in each skin biopsy is very small.

The full length HPV16-immortalized keratinocytes grown in Keratinocyte Growth Medium (KGM) as previously described (Mizrachy-Schwartz et al., 2007).

Primary fibroblasts were cultured from small biopsy specimens and grown in DMEM supplemented with 10% FBS, 100,000 U/l penicillin, 100 μg/l streptomycin.

Telomerase-negative normal human diploid foreskin fibroblast (BJ cells), BJ hTERT cells expressing a transfected hTERT (Bodnar et al., 1998), grown in DMEM supplemented with 10% FBS, 100,000 U/l penicillin, 100 μg/l streptomycin.

Retroviral Infection

Amphotropic retroviruses (PA317) expressing HPV-16 E6 and E7 genes were generated according to established procedures using the LXSN vector. The virus-producing cells were grown in DMEM supplemented with 10% fetal bovine serum (FBS) and the supernatant was collected from overnight cultures when cell were 80% confluent and stored at −70°C. Keratinocyte cell were grown without a feeder layer until they reached 80% confluence, then the medium was removed and 1ml of virus stock medium, containing 10μg/ml polybrene was added to each flask. Cells were infected for 2 hr, and then 5ml of the keratinocyte medium was added. Infected cells were selected in G418 (1000μg/ml) for 7 days and grew until they reached 80% confluency.

For *c-myc* expression we used the pWZL Blast myc or GFP retroviral plasmid. The plasmid was transiently transfected to Phoenix retroviral packaging cells. Keratinocyte cells were infected three times with the Phoenix cell supernatant, which contained the replication-defective virus. Then, the infected cells were selected using 6μg/ml of Blasticidin S, for the next 10 days.

For *cyclin E* expression we use pbabe-puro based vector which was a kindly provided by Professor J. Bartek.

LOH

Genomic DNA was extracted from primary keratinocytes and from keratinocytes expressing E6/E7 from the same donor, grown for 100 days, using a standard phenol/chloroform extraction procedure.

High-throughput micro-array genotyping of approximately 262,000 SNPs was performed using one array (version NspI) from the Affymetrix GeneChip Human Mapping 500K Array set (Affymetrix, Santa Clara, CA, USA). The recommended protocol, as described in the Affymetrix manual, was followed. Mapping array images were obtained using the GeneChip Scanner 3000 7G.

Immunofluorescence for Detection of γH2AX Foci

Primary keratinocytes were fixed in 3.7% formaldehyde/PBS for 10 min, permeabilized with 0.5% Triton/PBS, and blocked with 5% BSA/PBS. The primary antibodies used were mouse anti-γH2AX (Upstate Biotechnology). Appropriate Cy3 conjugated secondary antibodies were added (Jackson ImmunoResearch Laboratories). Images were taken with a Bio-Rad confocal microscope. For focus information analysis at least 50 nuclei for each condition were analyzed.

RT and Real-Time PCR

The effect of E6/E7 on the transcription level of genes from the nucleotide biosynthesis pathways was performed by reverse transcription and real time PCR. RNA was extracted using the RNeasy extraction kit (QIAGEN). RNA-less and reverse-transcriptase-less reactions were used as controls. Complementary DNA (cDNA) synthesis was performed as described previously (Rave-Harel et al., 1997). Real-time PCR was subsequently performed in ABI 7500 using a Power SYBR green PCR master Mix (Applied Biosystems). The expression level was normalized to the transcript levels of *POLR2A* and *GAPDH*. Specific primers for these PCR reactions were designed using the Primer Express software:

Glyceraldehyde 3-Phosphate Dehydrogenase

GAPDH: Fwd, TGAGCTTGACAAAGTGGTCG; Rev, GGCTCTCCAGAACATCATCC.

RNA Polymerase II

POLR2A: Fwd, TGCGCA CCATCAAGAGAGTC; Rev, CTCCGTCACAGACATTCGCTT.

E2F Transcription Factor 1

E2F1: Fwd, ACAGATCCCAGCCAGTCTCTACTC; Rev, GGACAACAGCGGTTCTTGCT.

Thymidylate Synthetase

TS: Fwd, GAATCACATCGAGCCACTGAAA; Rev, CAGCCCAACCCCTAAAGACTGA.

Ribonucleotide Reductase M2

RRM2: Fwd, ATGAAAACTTGGTGGAGCGATT; Rev, TGGCAATTTGGAAGCCATAGA.

Cyclin E1

Cyclin E1: Fwd, TTCTTGAGCAACACC CTCTTCTGCAGCC; Rev, TCGCCATATACC GGTCAAAGAAATCTTGTG CC.

Ribonucleotide reductase M2 B

RRM2B: Fwd, GGACAGCAGAAGAGGTCGACTTA; Rev, AAGCTTGTTCCAGTGAGGGAGA.

The primers used for amplification of *c-myc*, adenylosuccinate lyase (*ADSL*), pyridoxamine-pyruvate aminotransferase (*PPAT*), CTP synthetase (*CTPS*), nonmetastatic cells 1 (*NME1*), IMP (inosine monophosphate) dehydrogenase 2 (*IMPDH2*), and dihydroorotate dehydrogenase (*DHODH*) are as described previously (Liu et al., 2008).

Microarray Data Analysis

BJ cell expressing an empty vector, *cyclin E*, *c-myc*, or co-expressing both genes, were grown for 2-4 weeks. RNA was extracted from two sets of independent infected BJ cells using the RNeasy extraction kit (QIAGEN). Hybridization to GeneChip Human Gene 1.0 ST expression arrays, washing and scanning were performed according to the manufacturer's protocol (Affymetrix). Arrays were analyzed using RMA probeset condensation algorithm (Expression Console, Affymetrix). Genes were considered differentially expressed when the average change was over two-folds. We used the KEGG pathway. For the analysis of significantly changed pathways, the analysis was performed using the DAVID online functional annotation tool (http://david.abcc.ncifcrf.gov/) using default settings (Huang et al., 2009).

SUPPLEMENTAL REFERENCES

Bodnar, A.G., Ouellette, M., Frolkis, M., Holt, S.E., Chiu, C.P., Morin, G.B., Harley, C.B., Shay, J.W., Lichtsteiner, S., and Wright, W.E. (1998). Extension of lifespan by introduction of telomerase into normal human cells. Science *279*, 349–352.

Huang, W., Sherman, B.T., and Lempicki, R.A. (2009). Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat. Protoc. *4*, 44–57.

Liu, Y.C., Li, F., Handler, J., Huang, C.R., Xiang, Y., Neretti, N., Sedivy, J.M., Zeller, K.I., and Dang, C.V. (2008). Global regulation of nucleotide biosynthetic genes by c-Myc. PLoS ONE *3*, e2722.

Mizrachy-Schwartz, S., Kravchenko-Balasha, N., Ben-Bassat, H., Klein, S., and Levitzki, A. (2007). Optimization of energy-consuming pathways towards rapid growth in HPV-transformed cells. PLoS ONE *2*, e628.

Rave-Harel, N., Kerem, E., Nissim-Rafinia, M., Madjar, I., Goshen, R., Augarten, A., Rahat, A., Hurwitz, A., Darvasi, A., and Kerem, B. (1997). The molecular basis of partial penetrance of splicing mutations in cystic fibrosis. Am. J. Hum. Genet. *60*, 87–94.

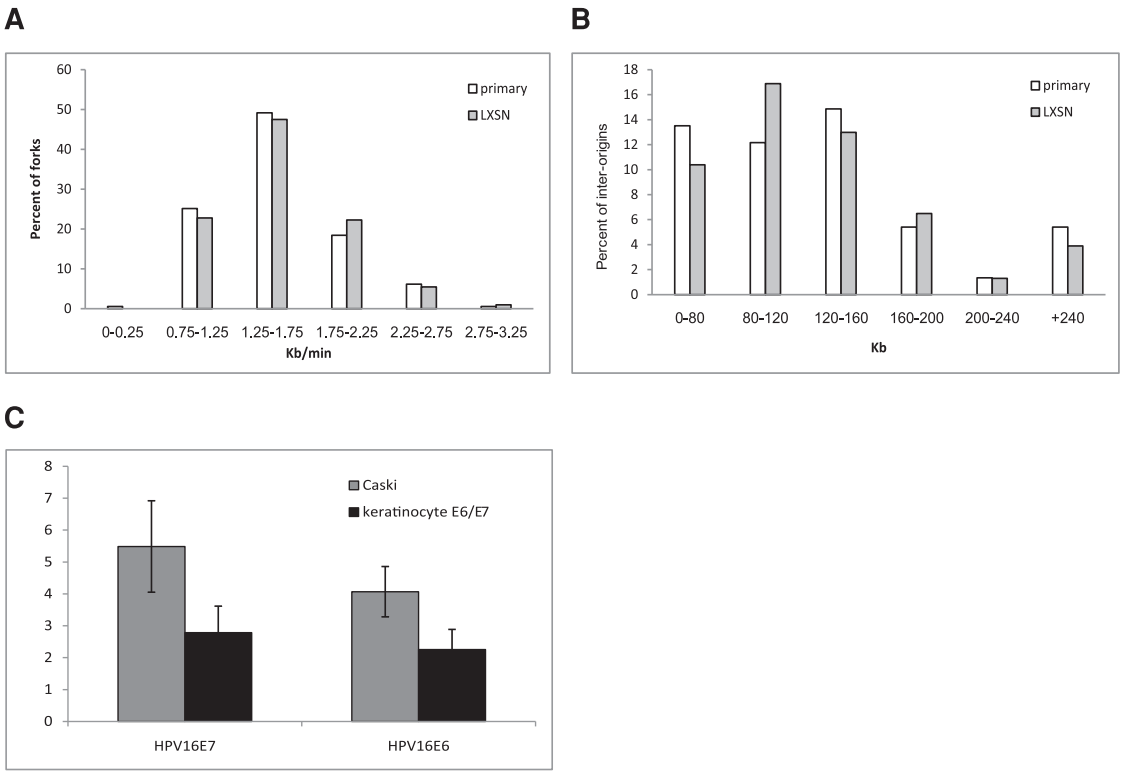

Figure S1. Characterization of Keratinocytes following Empty Vector and E6/E7 Expression, Related to Figure 1

(A) Fork rate distribution. White bars - primary (1 ± 0.3, n = 181), gray bars - primary expressing LXSN (1.1 ± 0.4, n = 203).

(B) Origin density distribution. White bars - primary (132 ± 13, n = 40), gray bars - primary expressing LXSN (129 ± 11, n = 42).

(C) RT-qPCR expression of the HPV-16 oncogenes E6 and E7 in keratinocyte cells expressing E6/E7 and in CasKi cells derived from HPV-16 induced cervical cancer. The expression was normalized to that of RPII.

The levels are expressed as mean fold change ± SEM, (n = 3).

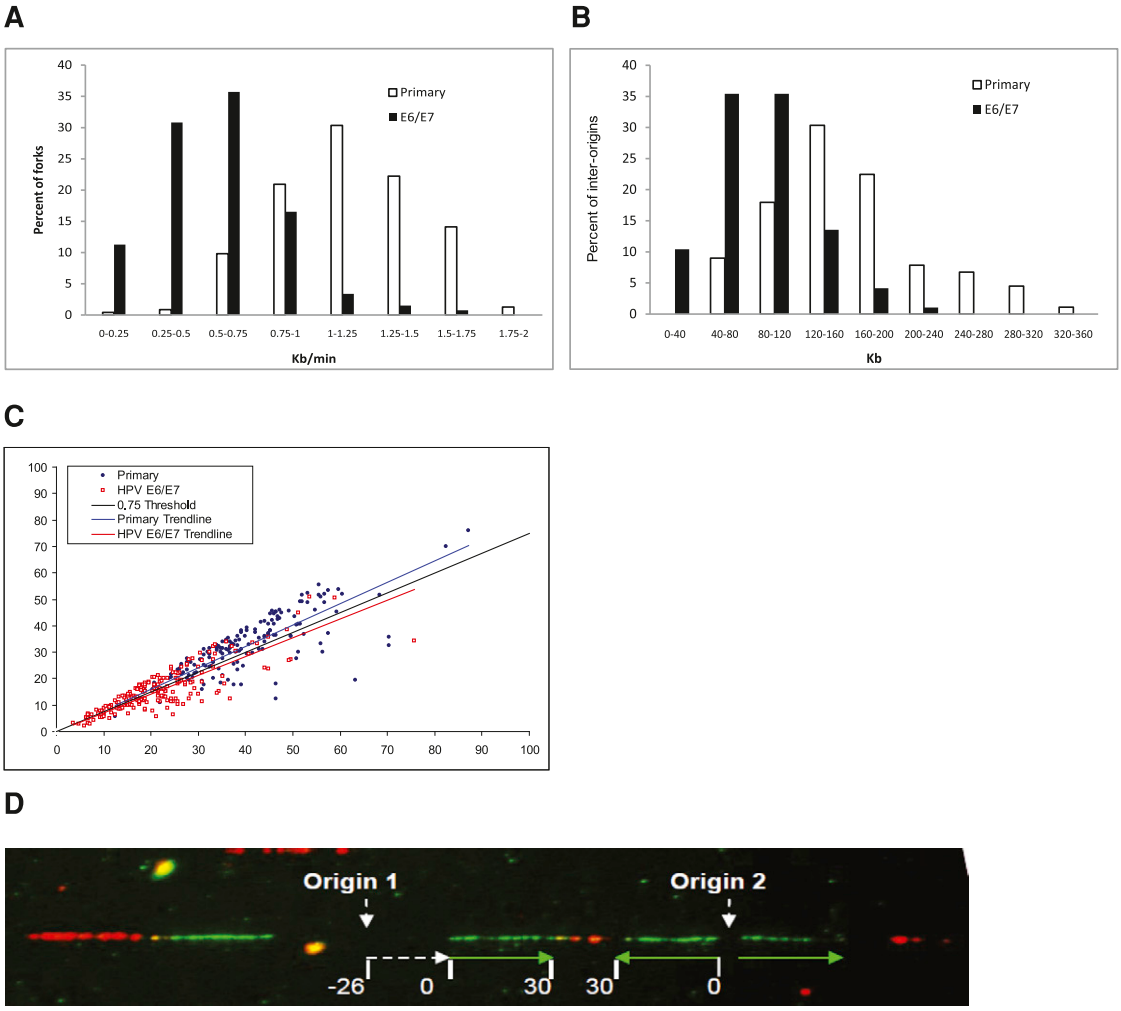

Figure S2. Replication Dynamics in Primary and HPV-16 E6/E7-Expressing Keratinocytes, Related to Figure 1

(A) Fork rate distribution. White bars - primary keratinocytes (1.1 ± 0.02 Kb/min, n = 235), black bars - keratinocytes expressing HPV-16 E6/E7 (0.57 ± 0.02 Kb/min, n = 267) ($p < 2*10^{-72}$).

(B) Origin density distribution. White bars - primary keratinocytes (392 ± 16 Kb, n = 90), black bars - keratinocytes expressing HPV-16 E6/E7 (218 ± 10, n = 97) ($p < 10*10^{-16}$).

(C) Correlated progression of left and right forks, emerging from the same origin for primary keratinocytes and keratinocytes expressing HPV-16 E6/E7.

(D) Example of a single combed DNA molecule with two adjacent replication origins. Origin 1 fired 26 min before the addition of IdU (green). Origin 2 fired close to the IdU addition. Numbers - the time in minutes relative to the addition of IdU.

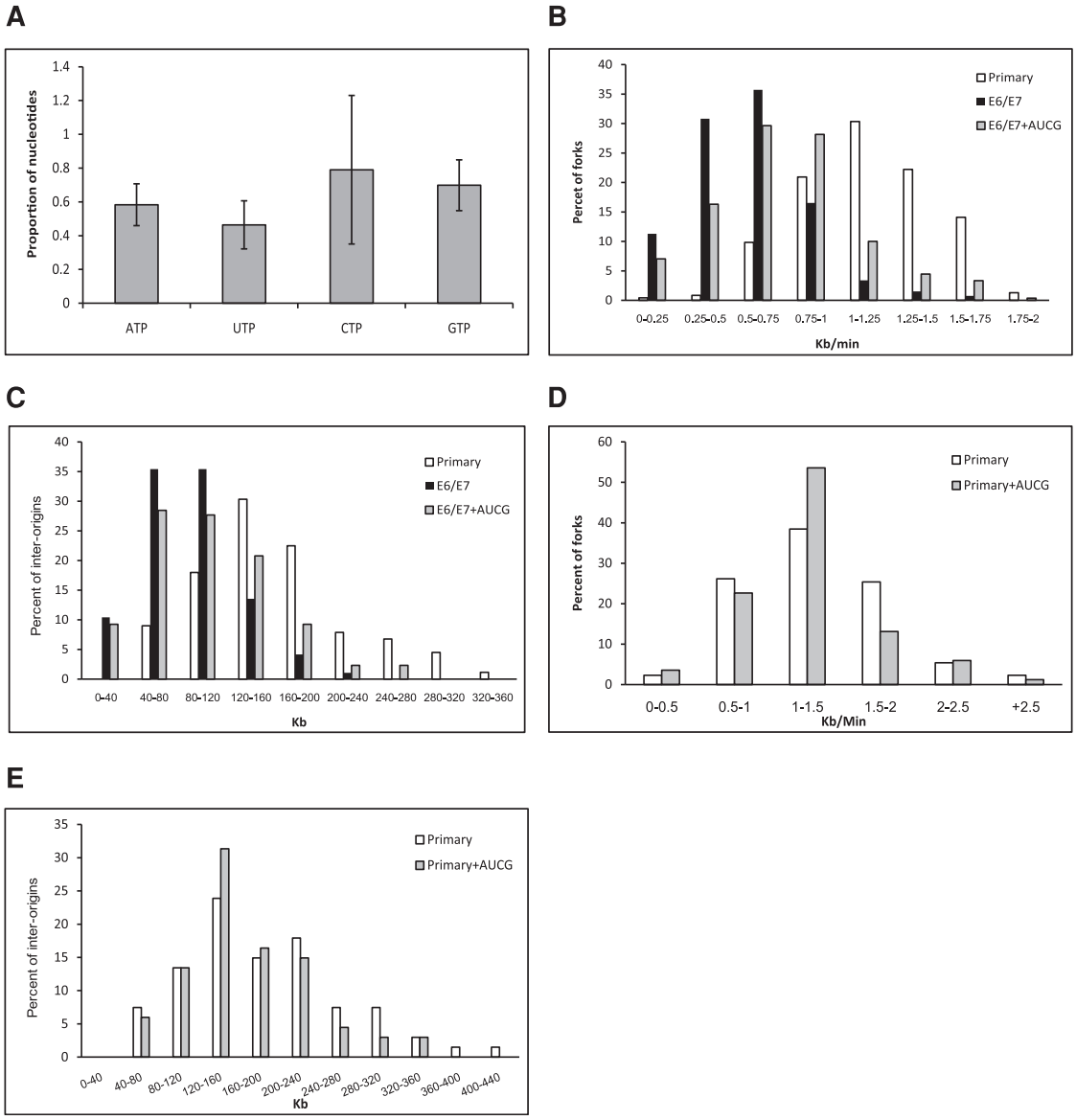

Figure S3. The Effect of Exogenous Supply of Nucleosides on the Replication Dynamics, Related to Figure 2

(A) rNTP levels of keratinocyte cells expressing HPV-16 E6/E7, normalized to primary keratinocytes. The levels are expressed as mean fold change ± SEM, (n > 3).

(B) Fork rate distribution. White bars - primary keratinocytes (mean 1.15 ± 0.02 Kb/min (n = 234)), black bars - keratinocytes expressing E6/E7 (mean 0.57 ± 0.02 Kb/min (n = 267)), gray bars - keratinocytes expressing E6/E7, grown for 48h with exogenous supply of nucleosides (mean 0.75 ± 0.02 Kb/min (n = 270)) ($p = 8*10^{-7}$).

(C) Origin density distribution. White bars - primary keratinocytes (mean 156 ± 17 Kb/min (n = 90)), black bars - keratinocytes expressing E6/E7 (mean 87 ± 10 Kb/min (n = 97)), gray bars - keratinocytes expressing E6/E7, grown for 48h with exogenous supply of nucleosides (mean 105 ± 12 Kb/min (n = 130)).

(D) Fork rate distribution. White bars - primary (1 ± 0.3, n = 181), gray bars - primary expressing LXSN (1.1 ± 0.4, n = 203).

(E) Origin density distribution. White bars - primary (132 ± 13, n = 40), gray bars - primary expressing LXSN (129 ± 11, n = 42).

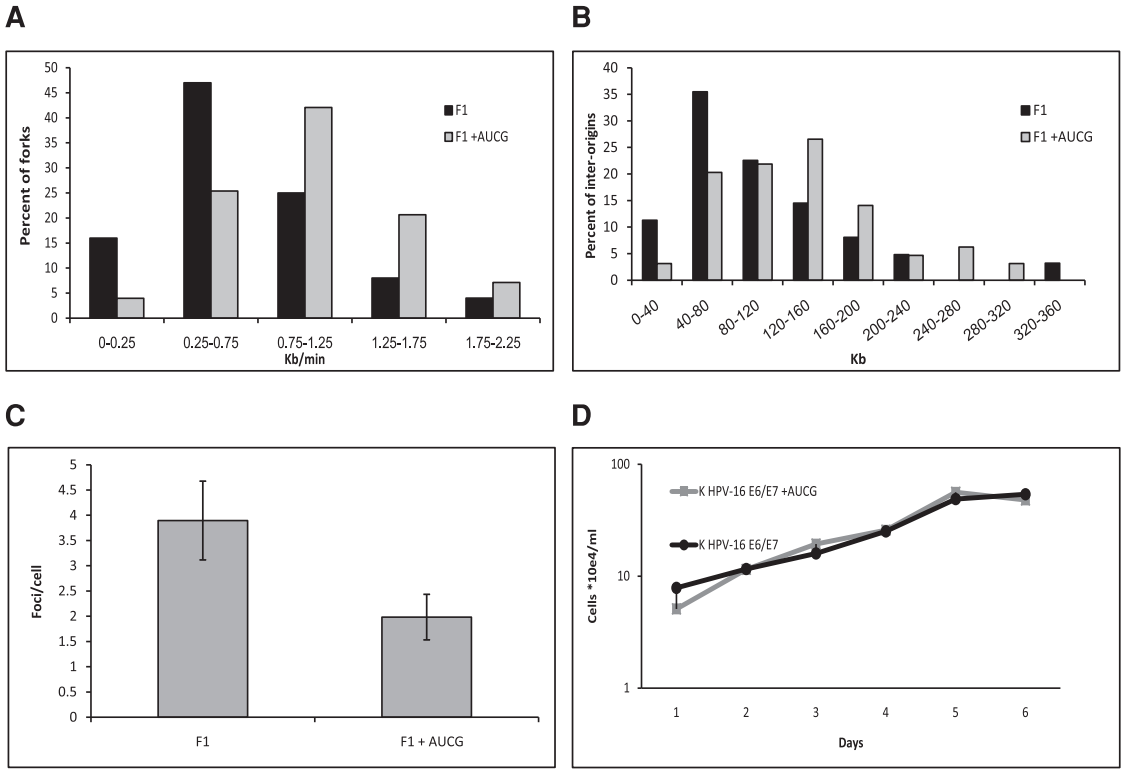

Figure S4. Characterization of Keratinocytes Expressing the Full-Length HPV-16 Genome and the Same Cells Grown with Exogenous Supply of Nucleosides, Related to Figure 2

(A) Fork rate distribution. Gray bars - keratinocyte cells expressing the full length HPV-16 genome (mean 0.69 ± 0.03 Kb/min (n = 42)), black bars - keratinocyte cells expressing the full length HPV-16 genome, grown for 48h with exogenous supply of nucleosides (mean 1.03 ± 0.04 Kb/min (n = 119)) ($p < 10^{*}10^{-10}$).

(B) Origin density distribution. Gray bars - keratinocyte cells expressing the full length HPV-16 genome (mean 106 ± 5 Kb (n = 65)), black bars - keratinocyte cells expressing the full length HPV-16 genome, grown for 48h with exogenous supply of nucleosides (mean 135 ± 8 Kb (n = 65)) ($p < 0.008$).

(C) The effect of exogenous supply of nucleosides on DNA damage. F1 - keratinocyte cells expressing the full length HPV-16 (mean 3.9 ± 0.4 foci per cell, n = 126). F1+AUCG - keratinocyte cells expressing the full length HPV-16, grown for 48h with exogenous supply of nucleosides (mean 1.9 ± 0.45 foci per cell, n = 61) ($p < 0.02$). The levels are expresses as mean number of foci per cell ± SEM).

(D) Proliferation rate of keratinocyte cells expressing HPV-16 E6/E7 and the same cells grown with nucleoside supply.

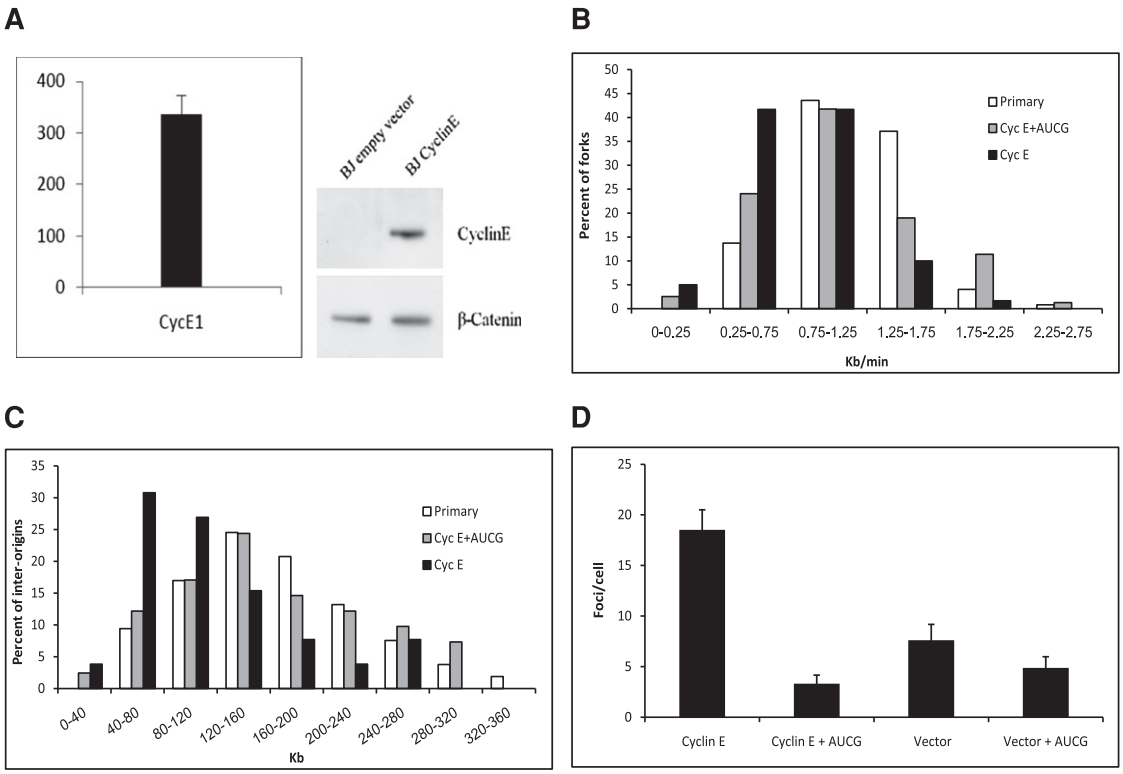

Figure S5. ***Cyclin E*** **Expression in Primary and BJ Fibroblast Cells and the Effect of Exogenous Nucleosides Supply, Related to Figure 3**

(A) Left - RNA levels in BJ cells expressing *cyclin E* normalized to the level of RPII using RT-qPCR. Right - protein levels using Western blot.

(B) Fork rate distribution. White bars - primary fibroblast cells infected with the empty vector (mean 1.2 ± 0.06 Kb/min (n = 125)), black bars - BJ cells expressing *cyclin E* (mean 0.8 ± 0.06 Kb/min (n = 62) ($p < 2*10^{-7}$)), gray bars - fibroblast cells expressing *cyclin E*, grown for 48h with exogenous supply of nucleosides (mean 1.1 ± 0.05 Kb/min (n = 80) (p = 0.0002)).

(C) Origin density distribution. White bars - primary fibroblast cells infected with the empty vector (mean 172 ± 12 Kb (n = 47)), black bars - fibroblast cells expressing *cyclin E* (127 ± 15 Kb (n = 27)) (p < 0.01), gray bars - fibroblast cells expressing cyclin E, grown for 48h with exogenous supply of nucleosides (mean 159 ± 11 Kb (n = 42)).

(D) Number of γH2AX foci per nuclei. Cyclin E – fibroblast cells from the same donor, expressing *cyclin E* (mean 18.5 ± 1.9 foci per cell, n = 39) ($p < 5*10^{-5}$), cyclin E + AUCG - fibroblast cells from the same donor expressing *cyclin E*, grown for 48h with exogenous supply of nucleosides (mean 3.3 ± 0.84 foci per cell, n = 44) ($p < 5*10^{-9}$). Vector - primary fibroblast cells infected with the empty vector (mean 7.7 ± 1.5 foci per cell, n = 35), vector + AUCG - primary fibroblast cells infected with the empty vector, grown for 48h with exogenous supply of nucleosides (mean 4.8 ± 1.1 foci per cell, n = 44).

The levels are expressed as mean number of foci per cell ± SEM.

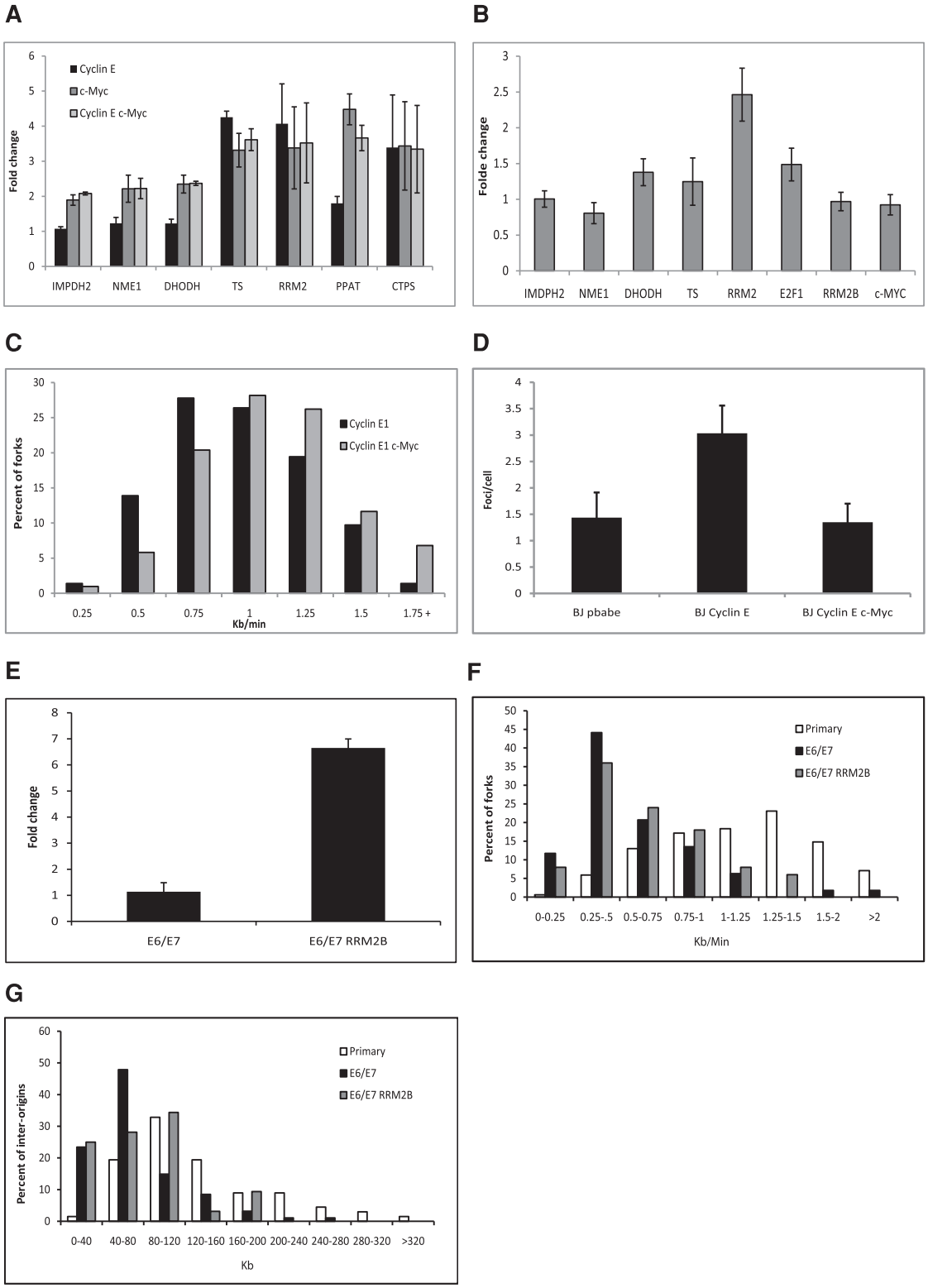

Figure S6. The Effect of *c-myc* and *RRM2B* Expression on Nucleotide Biosynthesis Genes and Replication Dynamics, Related to Figure 4 and Table 1

(A) Expression of genes involved in nucleotide biosynthesis following *cyclin E* and *c-myc* expression in BJ cells analyzed using HU_ST1 microarrays. The levels are expressed as mean fold change ± SEM, (n = 2).

(B) Expression of genes involved in nucleotide biosynthesis following *cyclin E* expression in BJ cells. The level of each analyzed transcript in *cyclin E* expressing BJ cells was measured using RT-qPCR and normalized to that of *RPII*. The ratio between the normalized levels was compared to the ratio in untreated BJ cells. The levels are expressed as mean fold change ± SEM, (n = 3).
(C) Fork rate distribution. Black bars - BJ cells expressing *cyclin E* (mean 0.84 ± 0.04 Kb/min (n = 72)), gray bars - BJ cells expressing *cyclin E*, with activation of the nucleotide biosynthesis by c-Myc (mean 0.97 ± 0.03 Kb/min (n = 104)) ($p < 0.005$).
(D) Number of γH2AX foci per nuclei. BJ - BJ cells infected with the empty vector (mean 1.4 ± 0.5 Foci/cell, n = 35), BJ cyclin E - BJ cells expressing *cyclin E* (mean 3.0 ± 0.5 Foci/cell, n = 64), BJ cyclin E c-Myc - BJ cells expressing *cyclin E* with activation of the nucleotide biosynthesis by *c-myc* expression (mean 1.3 ± 0.3 foci/cell, n = 48). The levels are expresses as mean number of foci per cell ± SEM). ($p = 0.009$).
(E) *RRM2B* expression in keratinocytes expressing E6/E7, relative to its expression in E6/E7 expressing cells, using RT-qPCR and normalized to that of *RPII*. The levels are expressed as mean fold change ± SEM, (n = 3).
(F) The effect of *RRM2B* and E6/E7 co-expression on fork rate distribution. White bars - primary keratinocytes (mean 1.22 ± 0.04 Kb/min (n = 169)), black bars - keratinocytes expressing E6/E7 (mean 0.54 ± 0.02 Kb/min (n = 161)), gray – keratinocytes co-expressing E6/E7 and *RRM2B* (mean 0.59 ± 0.04 Kb/min (n = 60)).
(G) The effect of *RRM2B* and E6/E7 co-expression on origin density distribution. White bars - primary keratinocytes (mean 132 ± 8 Kb (n = 67)), black bars - keratinocytes expressing E6/E7 (69 ± 4 Kb (n = 94)), gray bars - keratinocytes co-expressing E6/E7 and *RRM2B* (79 ± 7 Kb (n = 32)).