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Supplementary Information for

PTPN14 Degradation by High-Risk Human Papillomavirus E7 Limits Keratinocyte Differentiation and Contributes to HPV-Mediated Oncogenesis

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Other supplementary materials for this manuscript include the following:

Datasets S1 to S4

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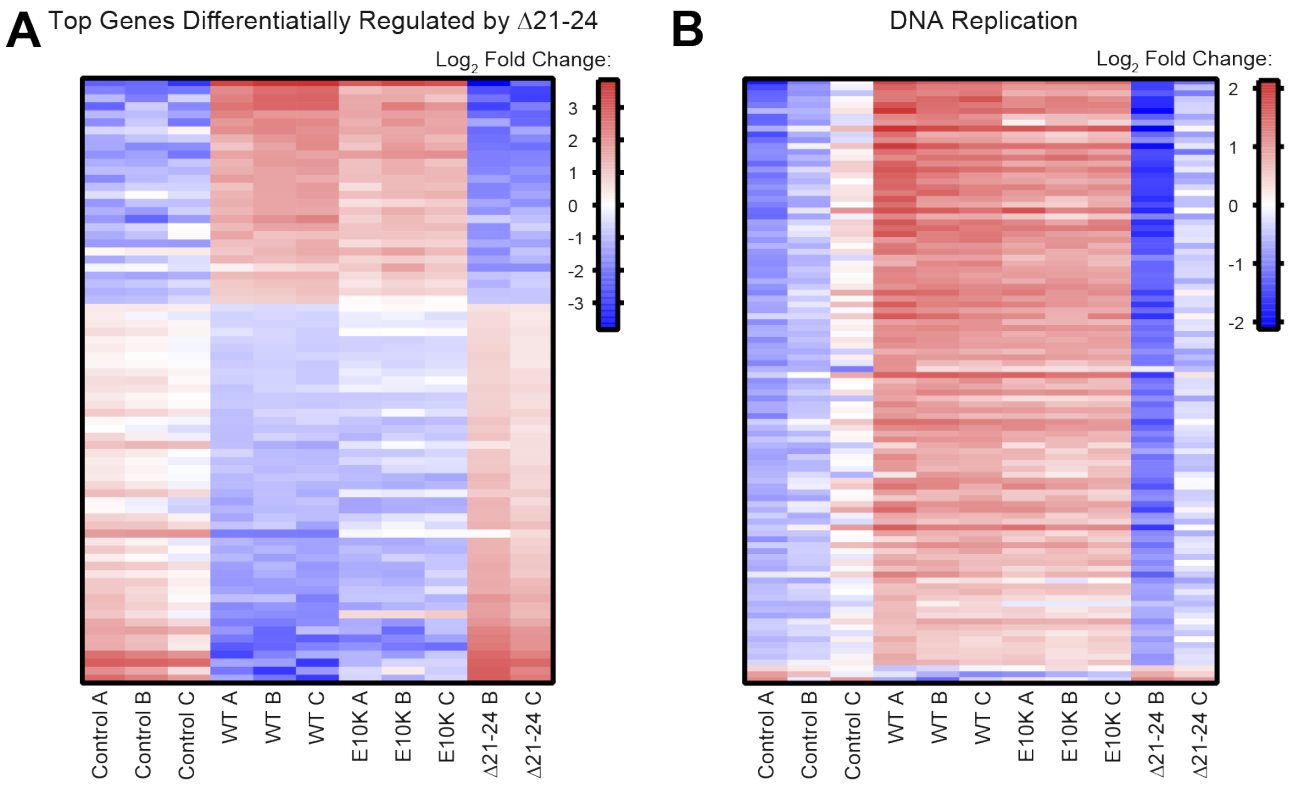

Fig. S1. The HPV16 E10K variant is impaired in PTPN14 degradation but binds RB1 and promotes cell cycle progression.

Primary HFK were transduced with retroviruses encoding HPV16 E7 WT, HPV16 E7 E10K, HPV16 E7 Δ21-24, or an empty vector control. RNA from transduced cells was polyA selected and analyzed by RNA-seq for changes in gene expression. (A) Heat map displays top 75 genes differentially expressed in HPV16 E7Δ21-24 relative to HPV16 E7 WT cells. (B) Genes in the DNA Replication GO Term (GO0006260) altered by HPV16 E7 WT relative to control ≥1.5 fold with p-value ≤0.05 are displayed in heat map.

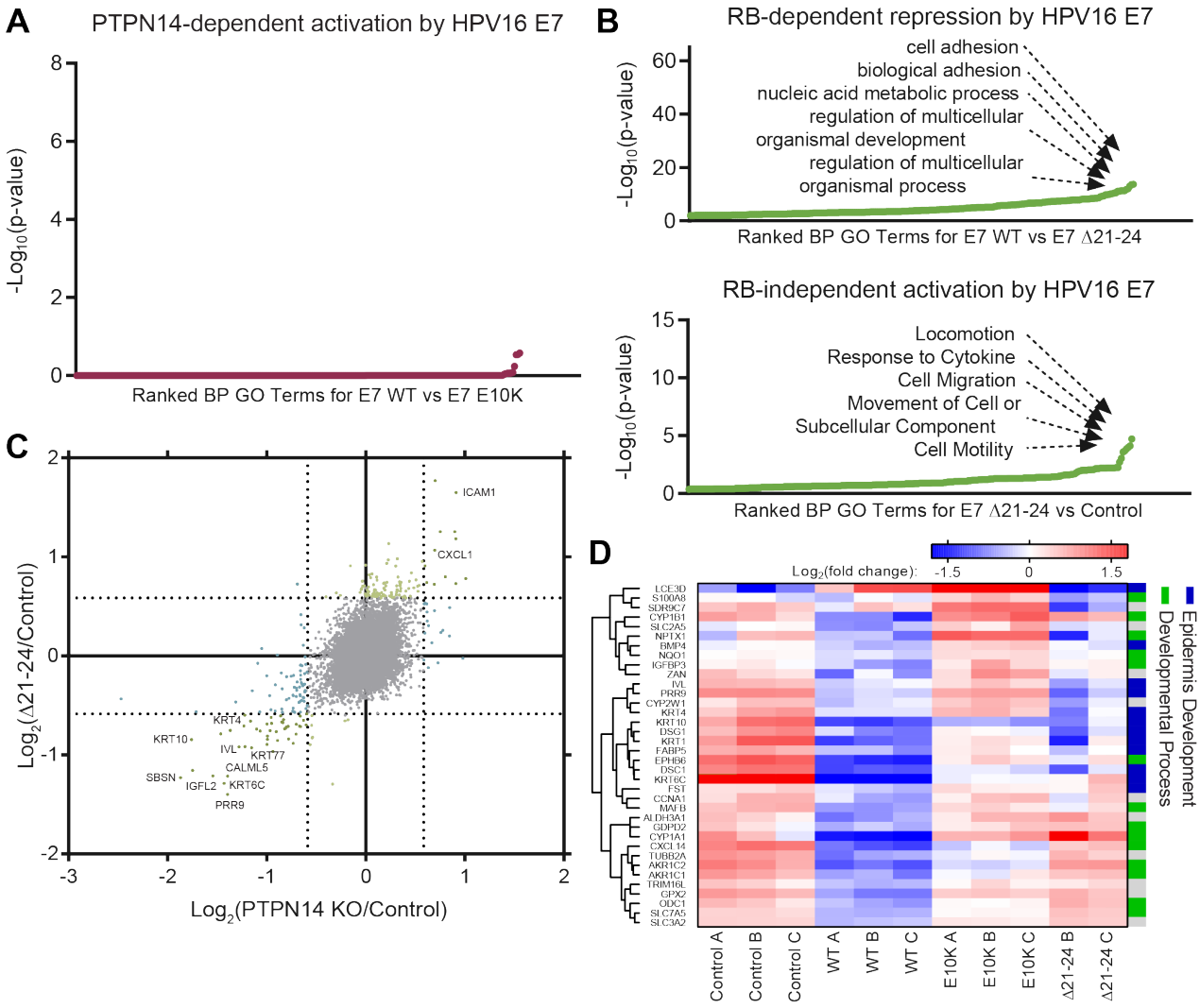

Fig. S2. HPV16 E7 degrades PTPN14 to inhibit keratinocyte differentiation.

Primary HFK were transduced with retroviruses encoding HPV16 E7 WT, HPV16 E7 E10K, HPV16 E7 Δ21-24, or an empty vector control. RNA from transduced cells was polyA selected and analyzed by RNA-seq for changes in gene expression. (A) GO enrichment analysis of genes >1.5 fold higher with p<0.05 in HPV16 E7 WT relative to HPV 16 E7 E10K shows no strong enrichment for GO terms. (B) Same analysis as (A) of (C, Top) genes with ≥1.5 fold lower expression in HPV16 E7 WT than HPV16 E7 Δ21-24 cells, and (C, Bottom) genes ≥1.5 fold higher expression in HPV16 E7 Δ21-24 cells relative to empty vector control cells and *p*-value ≤0.05. (C) Scatter plot of $\log_2$(fold-change) in gene expression compares the gene expression changes of HPV16 E7 Δ21-24 relative to empty vector control to those of PTPN14 KO relative to control. Colors denote whether genes are altered by PTPN14 KO only (blue), by HPV16 E7 Δ21-24 only (light green), or both (dark green). (D) Unbiased clustering of genes that are lower in HPV16 E7 WT cells relative to HPV16 E7 E10K by ≥1.5 fold with *p*-value ≤0.05 in HPV16 E7. Gene names and clustering are displayed to the left of the heat map, and selected GO categories are displayed on the right. Color coding on the right side denotes whether genes in a cluster are related to epidermis development (blue), other developmental processes (green), or neither (gray).

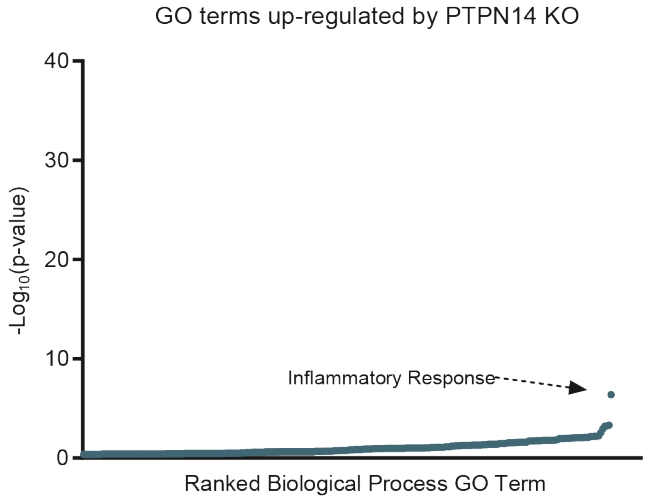

Fig. S3. PTPN14 depletion upregulates inflammatory response genes in primary human keratinocytes and down regulates similar keratinocyte differentiation genes to HPV16 E7.

Primary HFK were transduced with LentiCRISPRv2 lentiviral vectors encoding SpCas9 and non-targeting or PTPN14-directed sgRNAs and analyzed for changes in gene expression. PolyA selected RNA was analyzed by RNA-seq. Plot displays GO enrichment analysis of genes upregulated in HFK-PTPN14 KO compared to HFK-control.

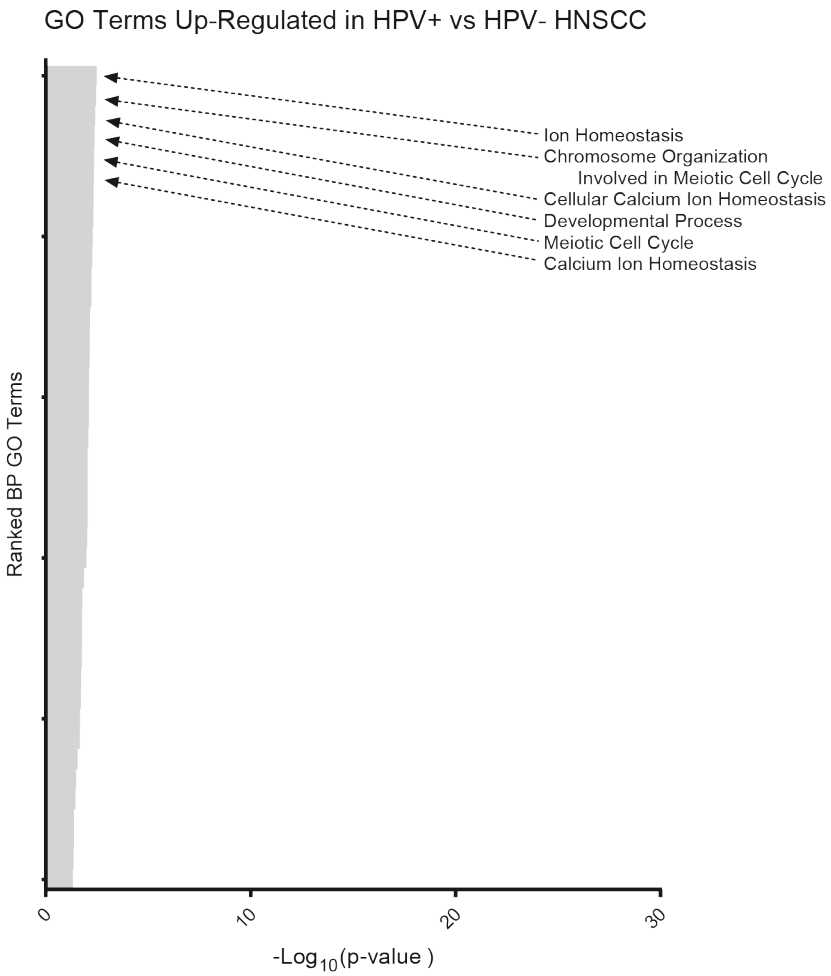

Fig. S4. Keratinocyte differentiation gene signature describes the major differences between HPV+ and HPV- HNSCC.

Data from HNSCC samples on the TCGA database were determined to be HPV+ or HPV- and analyzed for differences in gene expression. (A) Bar chart portrays the ranked $-Log_{10}$(p-values) of enriched gene ontology terms among genes up-regulated in HPV+ HNSCC.

Dataset S1 (separate file)

The HPV16 E10K variant is impaired in PTPN14 degradation but binds RB1 and promotes the expression of E2F-regulated genes.

Primary HFK were transduced with retroviruses encoding HPV16 E7 WT, HPV16 E7 E10K, HPV16 E7 Δ21-24, or an empty vector control. RNA from transduced cells was polyA selected and analyzed by RNA-seq for changes in gene expression. (A) Table includes top 75 genes significantly altered by HPV16 E7 Δ21-24 relative to HPV16 E7 WT. (B) Table displays the genes in the DNA Replication GO Term (GO0006260) that are altered by ≥1.5 fold with p-value ≤0.05 in HPV16 E7 WT cells relative to vector control cells. Tables include gene name, log2(fold change), and adjusted p-value for HPV16 E7 E10K and HPV16 E7 Δ21-24 relative to HPV 16 E7 WT.

Dataset S2 (separate file)

HPV16 E7 degrades PTPN14 to inhibit keratinocyte differentiation.

Primary HFK were transduced with retroviruses encoding HPV16 E7 WT, HPV16 E7 E10K, HPV16 E7 Δ21-24, or an empty vector control. RNA from transduced cells was polyA selected and analyzed by RNA-seq. (A) Table includes gene name, log2(fold change), and adjusted p-value for genes altered by ≥1.5 fold with *p*-value ≤0.05 in HPV16 E7 WT relative to HPV16 E7 E10K. (B) Table includes gene name, log2(fold change), and adjusted p-value for genes altered by ≥1.5 fold with *p*-value ≤0.05 in HPV16 E7 Δ21-24 relative to empty vector control.

Dataset S3 (separate file)

PTPN14 depletion impairs differentiation-related gene expression in primary human keratinocytes.

Primary HFK were transduced with LentiCRISPRv2 lentiviral vectors encoding SpCas9 and non-targeting or PTPN14-directed sgRNAs and polyA selected RNA was analyzed by RNA-seq. Table includes gene name, $\log_2$(fold change), and adjusted p-value for genes differentially expressed by ≥1.5 fold with p-value ≤0.05.

Dataset S4 (separate file)

Plasmids, primers, and siRNAs used in the study.