

Figure S1: CircE7 Expression and Outcomes of VSCC by CircE7 and P16.

(a) Sanger sequencing confirmed the expected backsplice junction for HPV16 circE7. (b) Representative PCR products from VSCC samples for circE7 (~150 base pairs). Controls represent VSCC samples that were previously positive or negative. The presence of multiple bands indicates the presence of distinct circE7 isoforms generated by multiple backsplice events. (c) Actin was used as an internal control (~100 base pairs). (d) Kaplan–Meier curves for overall-survival and progression-free survival based on presence or absence of circE7 expression and compared by log-rank test with a p-value <0.05 considered significant. (e) Kaplan–Meier curves for overall-survival and progression-free survival by p16 staining and compared by log-rank test with a p-value <0.05 considered significant.

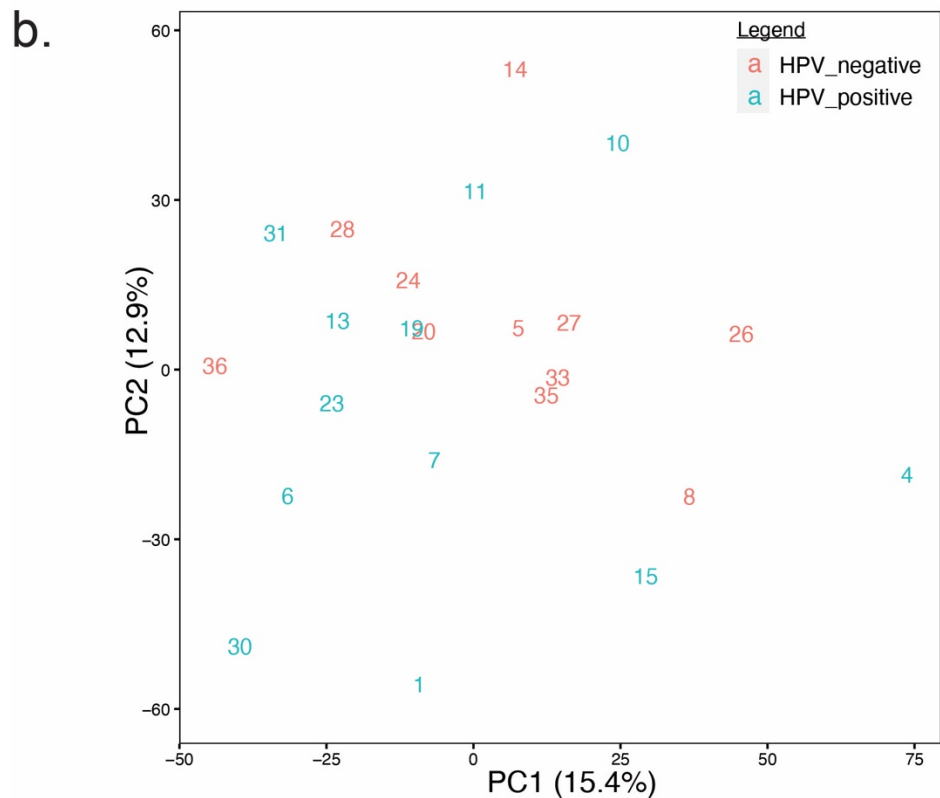
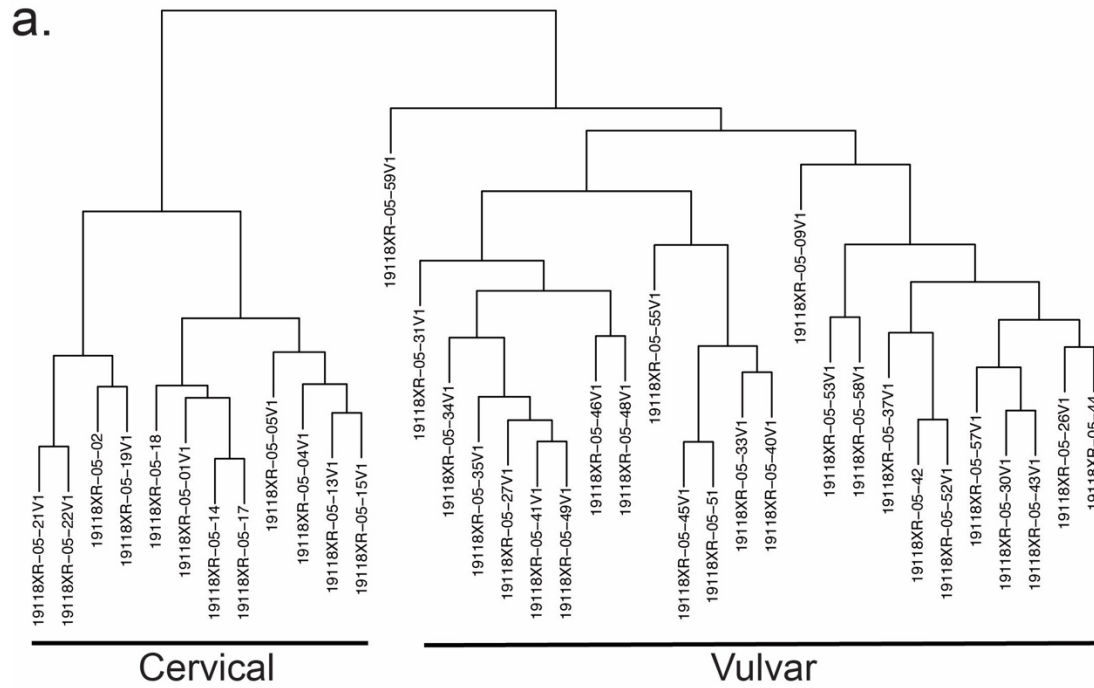


Figure S2. Clustering analyses of RNA-Seq.

(a) Complete hierarchical clustering analysis from RNA-sequencing for VSCC and cervical squamous cell carcinoma samples (CSCC). VSCC and CSCC clustered independently in unsupervised hierarchical clustering. (b) Principal components analysis of VSCC sequencing. Colors indicate consensus HPV-positive and HPV-negative groups.

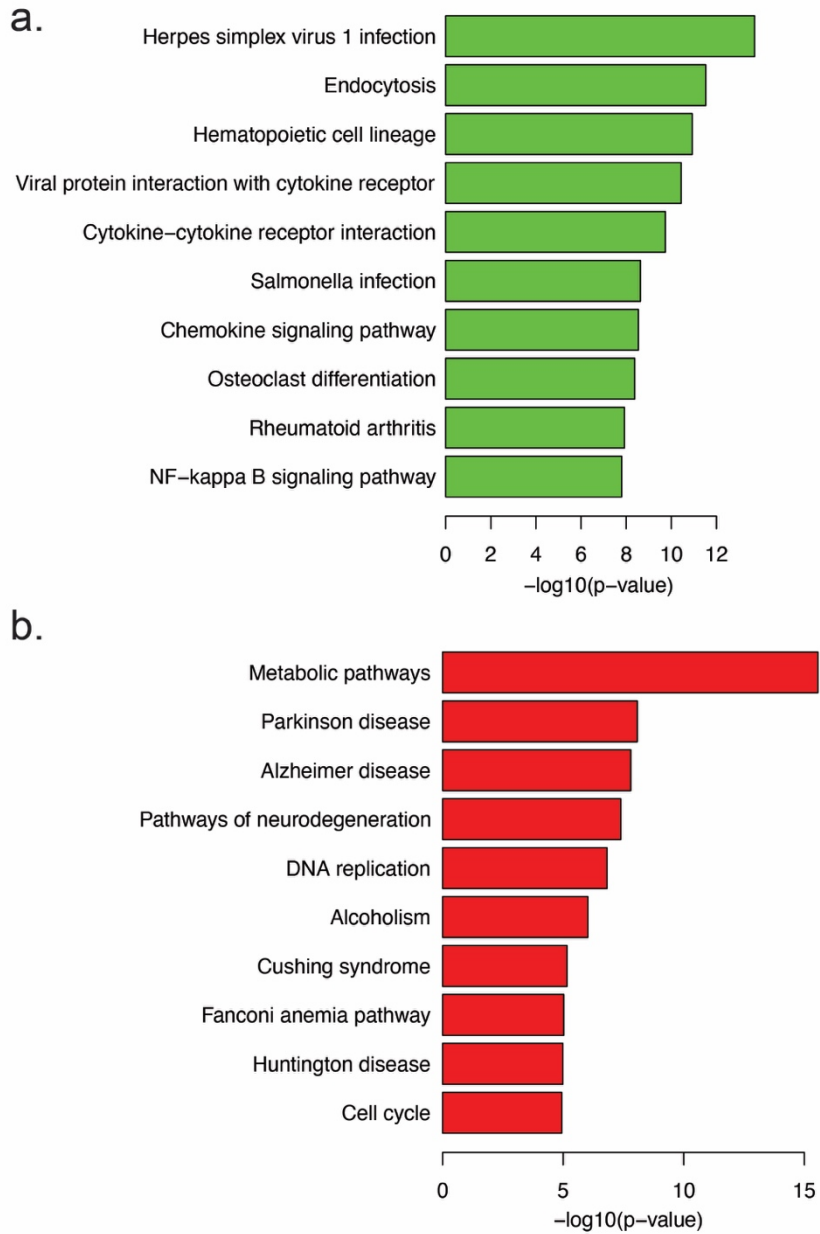


Figure S3. Clustering analyses of RNA-Seq.

KEGG pathways analysis revealed the most significantly (a) upregulated and (b) downregulated pathways in HPV-positive vs HPV-negative VSCC.

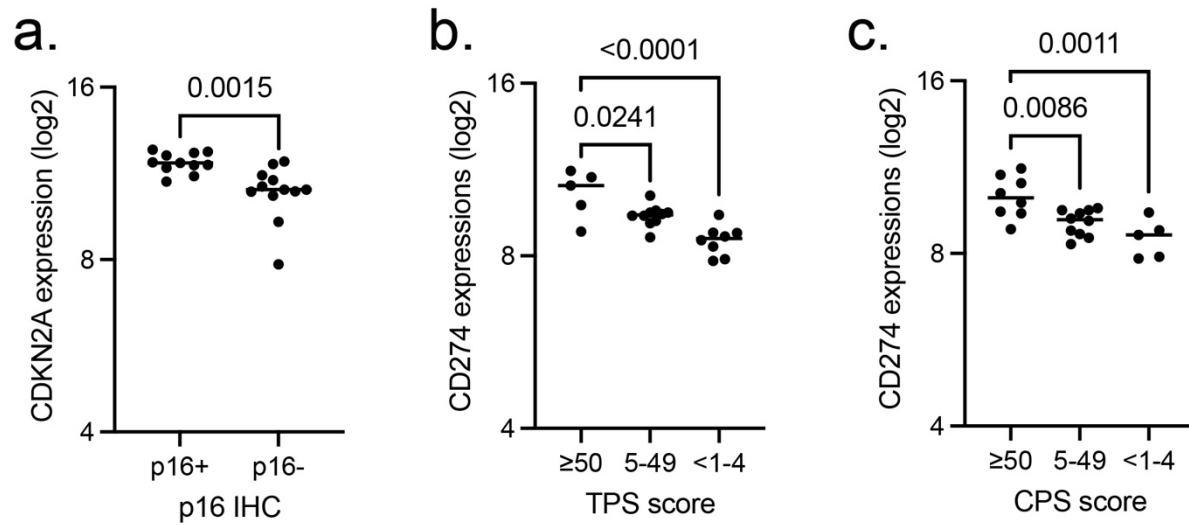


Figure S4. Correlation of RNA-Seq with IHC staining results.

Comparison of immunohistochemical (IHC) staining analyses with RNA-Seq expression data. (a) CDKN2A expression is significantly lower in p16 IHC negative cases. (b) CD274 expression correlates with PD-L1 TPS score. (c) CD274 expression correlates with PD-L1 CPS score.

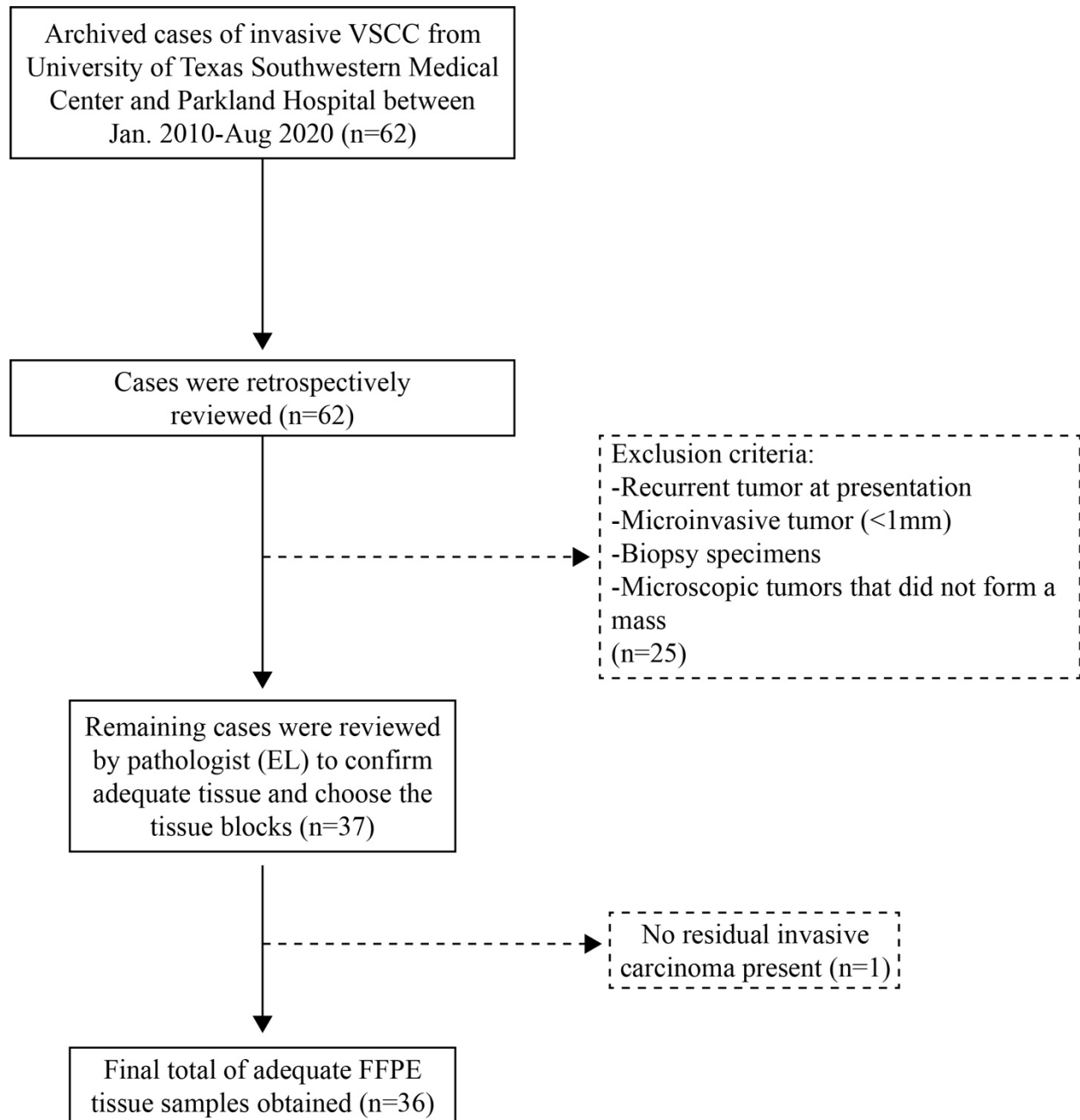


Figure S5: Inclusion and Exclusion Criteria for Selection of Vulvar Squamous Cell Carcinoma Samples.

Inclusion and exclusion criteria used for the selection of vulvar squamous cell carcinoma samples for subsequent analysis.

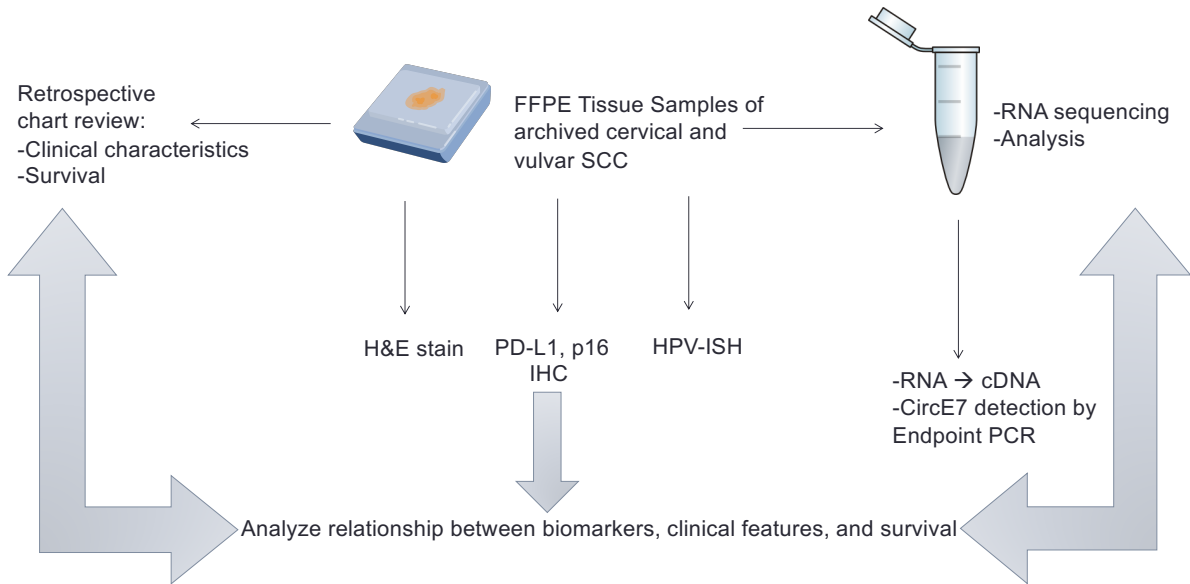


Figure S6: Diagram of Study Design.
Schematic of the study design for this project.