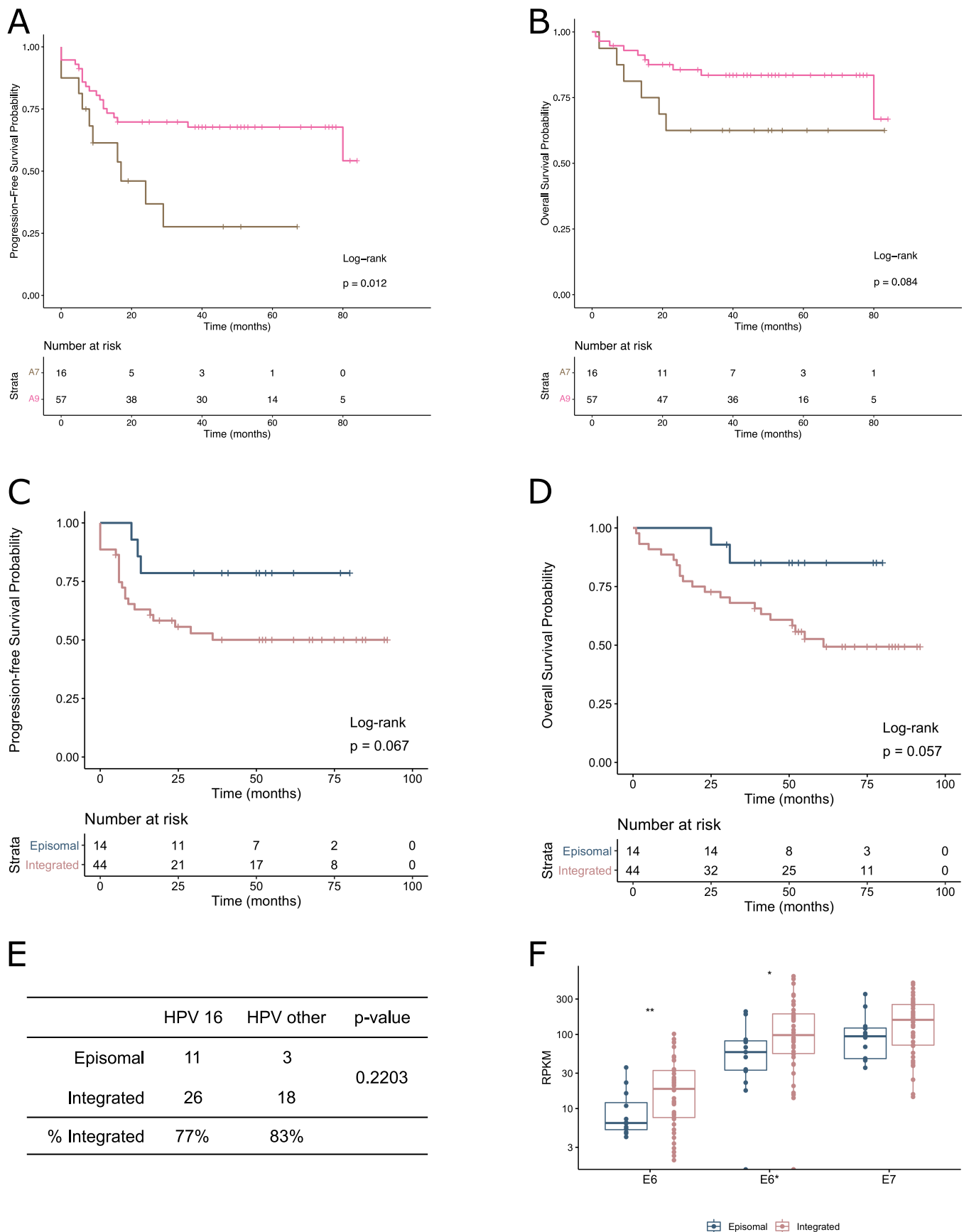




Supplementary Figure 1: Association of viral clade and state with patient outcomes and HPV transcript expression

Progression – free (A,C) and overall survival curves (B,D) stratified by viral clade ($n = 36$) and viral genome integration ($n = 58$). (A-D Log rank test was used to determine statistical significance, calculated using the survminer package in R version 3.5.2. E) Episomal and integrated samples by HPV genotype group (Fisher exact). F) HPV E6, E6* and E7 transcript expression (RPKM) by viral state (Wilcoxon signed-rank test).

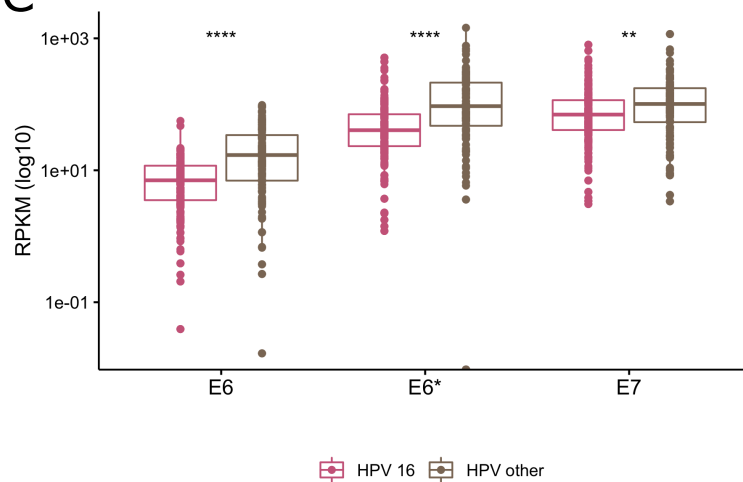
A

TCGA HPV Genotype	n = 309
HPV 16	174
HPV 18	40
HPV other	93
NA	2

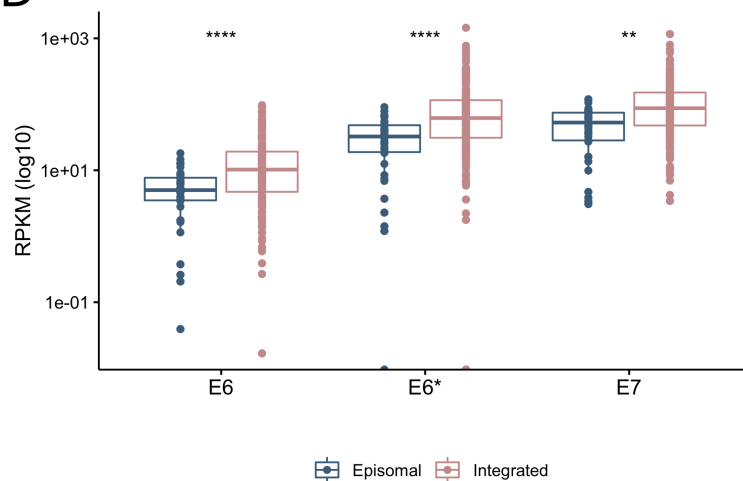
B

	HPV 16	HPV other	p-value
Episomal	40	23	0.2548
Integrated	134	110	
% Integrated	70%	86%	

C



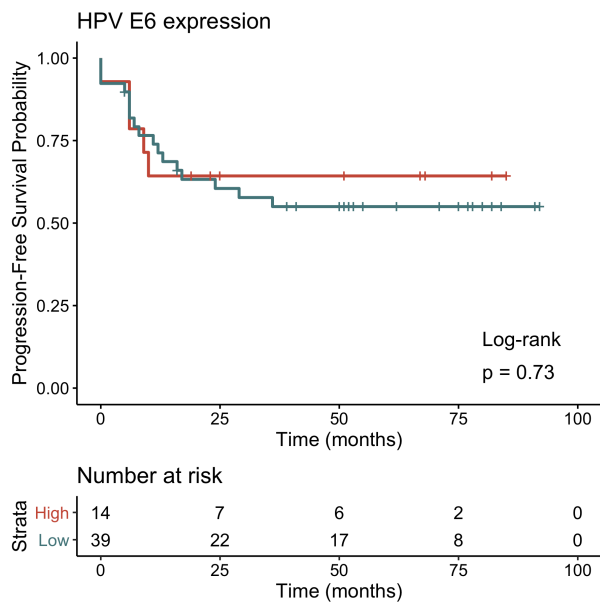
D



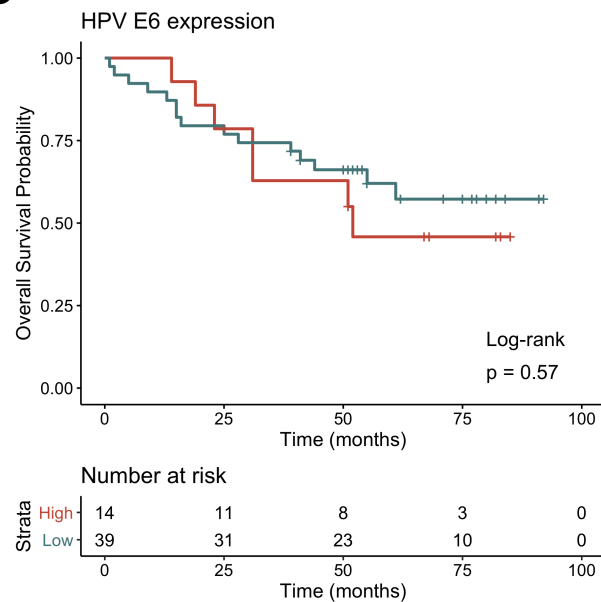
Supplementary Figure 2: TCGA viral state and HPV transcript expression

A) HPV genotypes and B) viral integration (chi-square test). C-D) HPV E6, E6* and E7 transcript expression (RPKM) by C) HPV genotype and D) viral state (Wilcoxon signed-rank test).

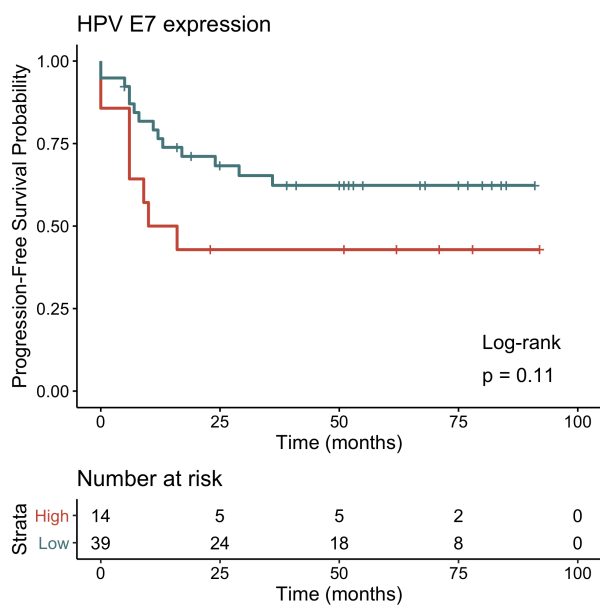
A



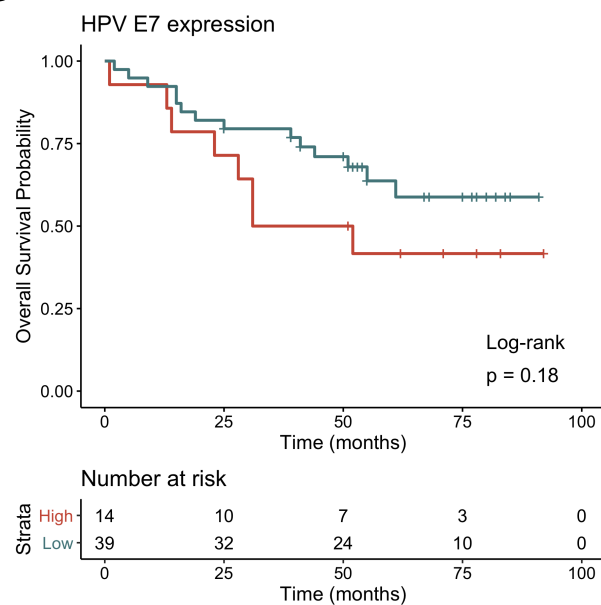
B



C



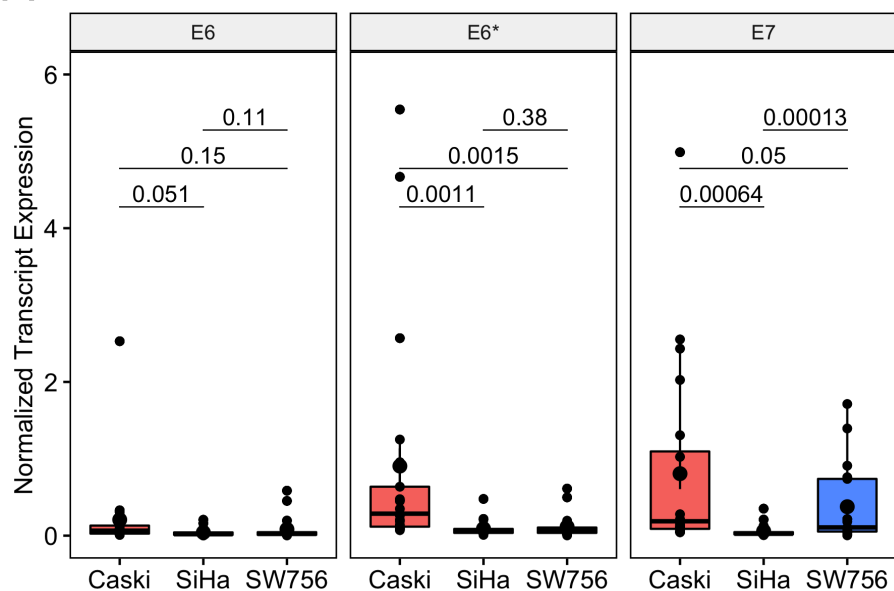
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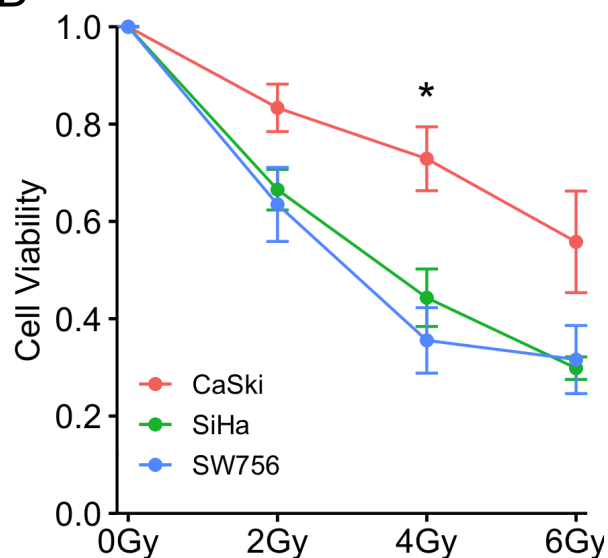
Supplementary Figure 3: Patient outcomes stratified by relative HPV transcript expression

Progression-free and overall survival was assessed for patients grouped based upon relative HPV transcript expression, high > Q3 and low < Q3 for A-B) HPV E6, and C-D) HPV E7 (Log rank test was used to determine statistical significance, calculated using the survminer package in R version 3.5.2)

A

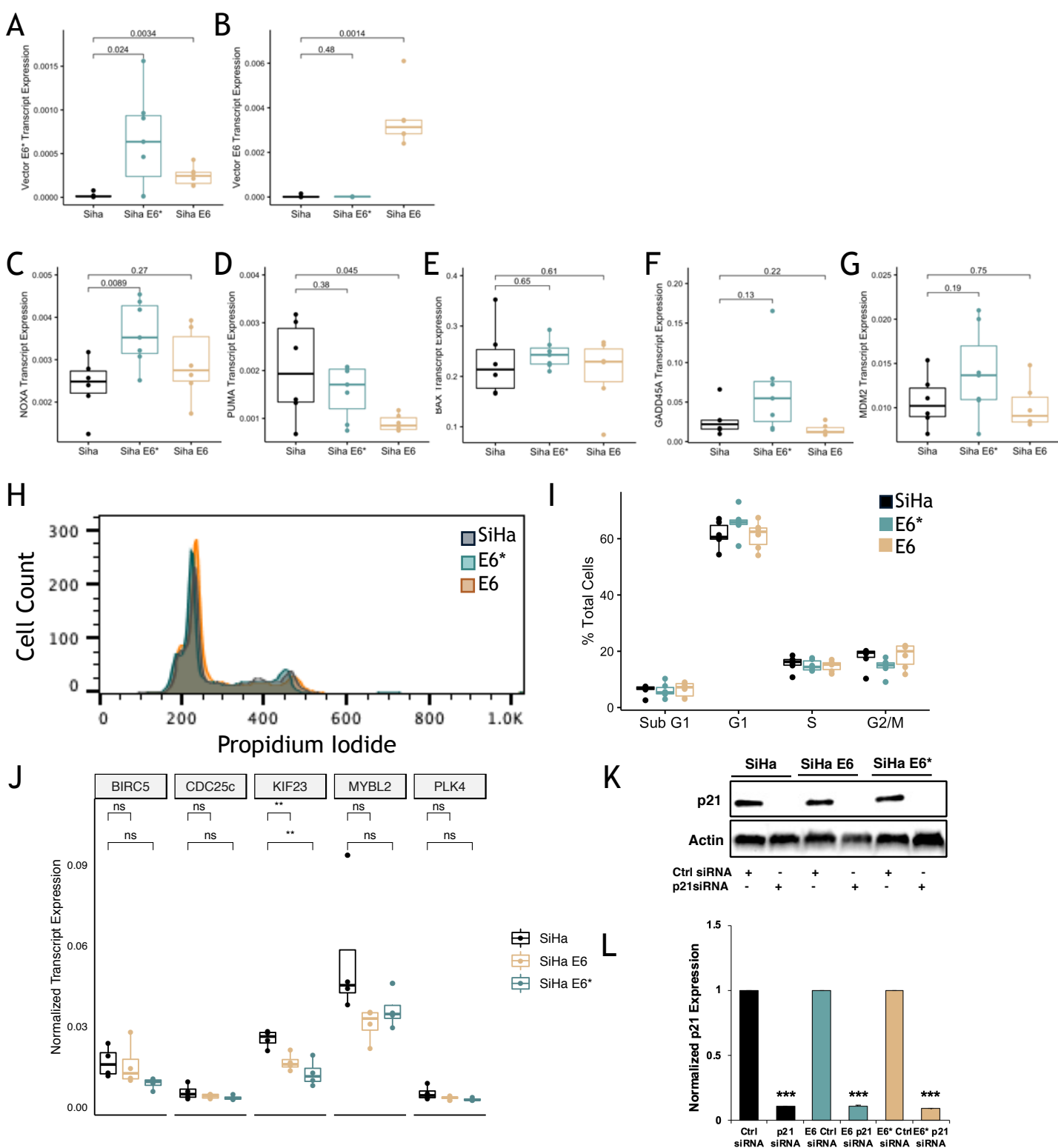


B



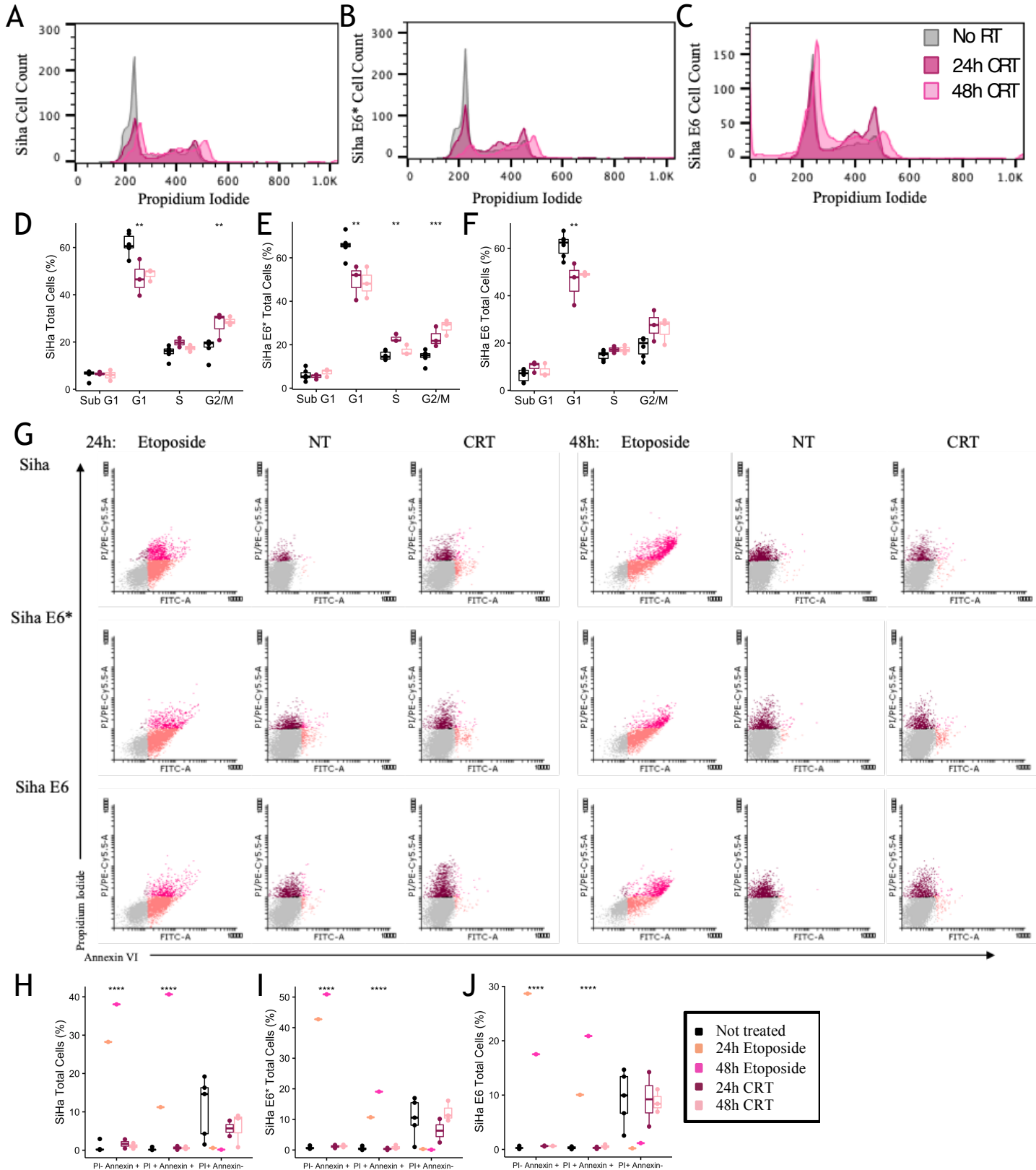
Supplementary Figure 4: Cervix cancer cell lines transcript expression and sensitivity to radiation treatment

A) CaSki, SiHa and SW756 relative HPV transcript expression by qRT-PCR (Normalized to GAPDH and ACTB transcript expression). (Data from 3 independent experiments, students t-test) B) Cell line sensitivity to increasing doses of radiation treatment assessed by alamarBlue. (Mean $\pm$ SEM, data from 3 independent experiments, one-way ANOVA).



Supplementary Figure 5: Generation of HPV E6* and E6 overexpressing cell lines and their effect on p53 and p21

A,B) HPV vector specific expression of HPV 16 E6* and E6 across the cell lines. C-G) p53 target gene expression. (A-G Normalized to GAPDH and ACTB transcript expression, n = 6, students t-test). H-I) Basal cell cycle phase and quantification assessed by flow cytometry, no significant differences were observed across the cell lines tested (n = 3, one-way ANOVA). J) DREAM target gene expression (Normalized to GAPDH, n = 3, students t-test). K-L) Transient siRNA knockdown of p21 across the cell lines. (Normalized to Actin, lanes run on same gel but were not contiguous. n = 3, students t-test).



Supplementary Figure 6: E6* and E6 overexpression does not alter G2/M cell cycle arrest following CRT treatment and apoptosis not cell death mechanism post-CRT treatment

A-F) Cell cycle analysis by flow 24 and 48hrs after 0.5 μ M carboplatin and 4Gy radiation treatment. (dark pink = 24h, light pink = 48h). Representative flow and quantification of SiHa, SiHa E6* and SiHa E6 (n = 3, one-way ANOVA). G-J) Apoptosis analysis by flow 24 and 48hrs after 0.5 μ M carboplatin and 4Gy radiation treatment. (dark pink = 24h, light pink = 48h). G) Representative flow plots of Annexin V and Propidium iodide staining in SiHa, SiHa E6* and SiHa E6 using etoposide as a positive control for apoptosis, H-J) SiHa, SiHa E6* and SiHa E6 quantified staining (n = 3, one-way ANOVA).

Supplementary Table 1: HPV E6, E6* and E7 primer sequences

Native Sequences			
Genotype	Gene	Primer Sequence	Reference
HPV 16	E6	F 5' - GCGACGTGAGGTATATGACTTT	7
		R 5' - TCAGGACACAGTGGCTTTT	
	E6*	F 5' - ACTGCAATGTTTCAGGACCCA	7
		R 5' - TAATACACCTCACGTGCAG	
	E7	F 5' - AAGTGTGACTCTACGCTTCGGTT	34
		R 5' - GCCCATTAACAGGTCTTCCAAA	
HPV 18	E6	F 5' - ATCCAACACGGCGACCCTAC	35
		R 5' - GCACCGCAGGCACCTTATTA	
	E6*	F 5' - TTGGAACTTACAGAGGTGCC	36 ^a
		R 5' - CGAATGGCACTGGCCTCTATAG	
	E7	F 5' - TAATCATCAACATTTACCAGCCCG	36
		R 5' - CGTCTGCTGAGCTTTCTACTACTA	
Vector Sequences			
Genotype	Gene	Primer Sequence	
HPV 16	E6	F 5' - GAAGTGACGATTTTCGCTT	
		R 5' - GTGAGTAACAATAGTGCCT	
	E6*	F 5' - GTGTACTAATTCGCTTTTCAG	
		R 5' - GTGAGTAACAATAGTGCCT	

^aAdapted from HPV 18 E6 R primer