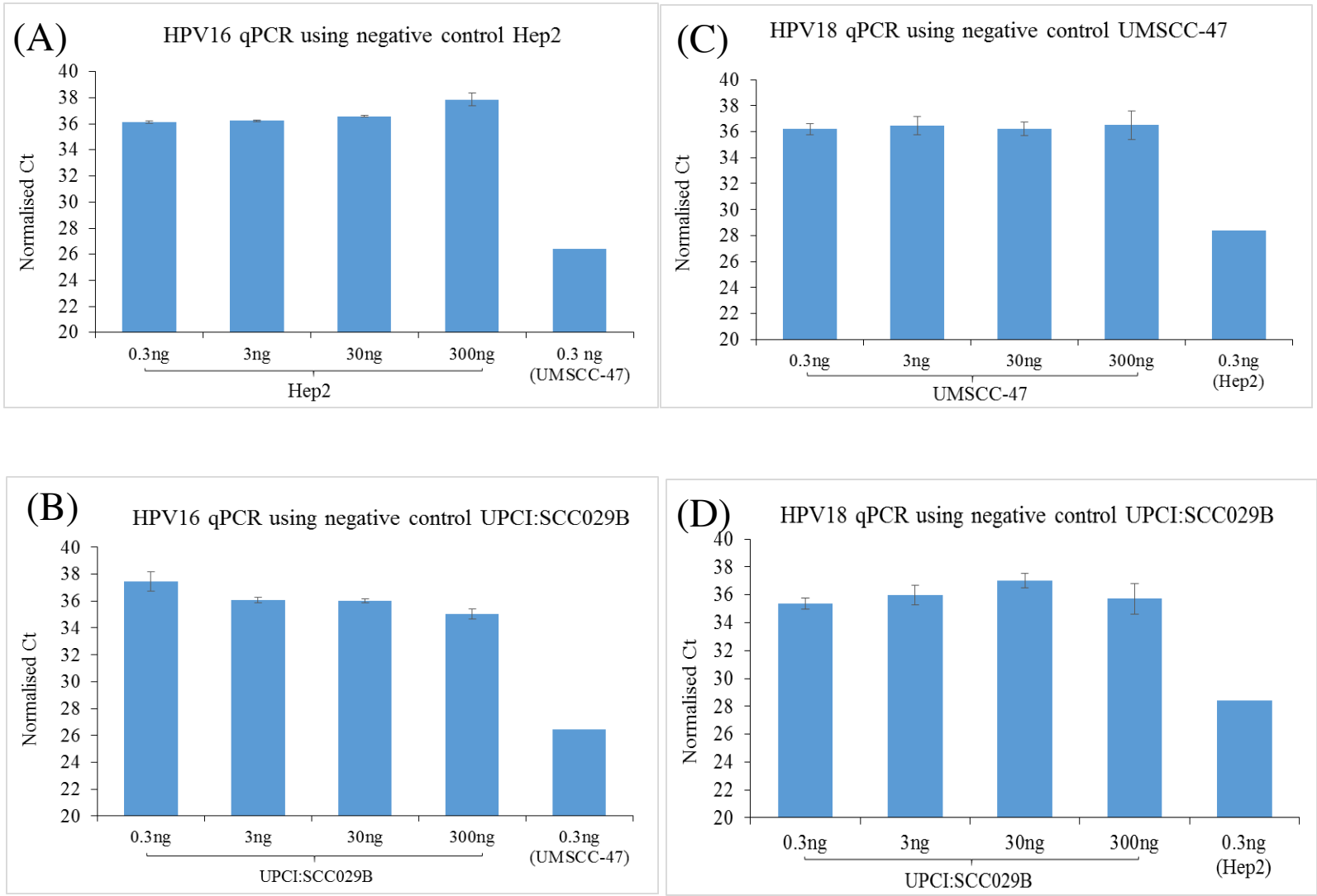
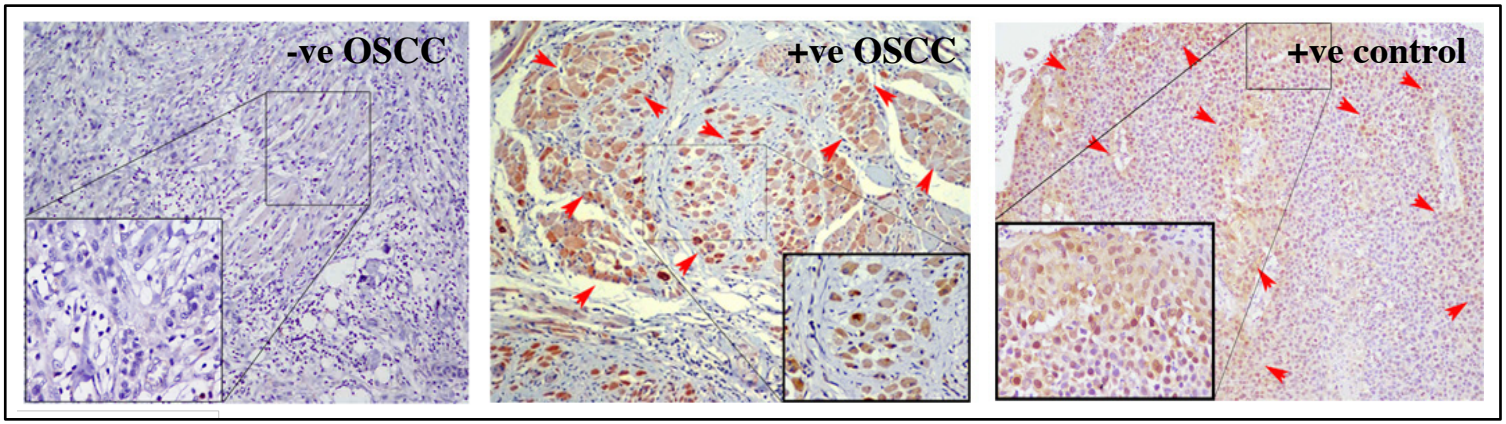
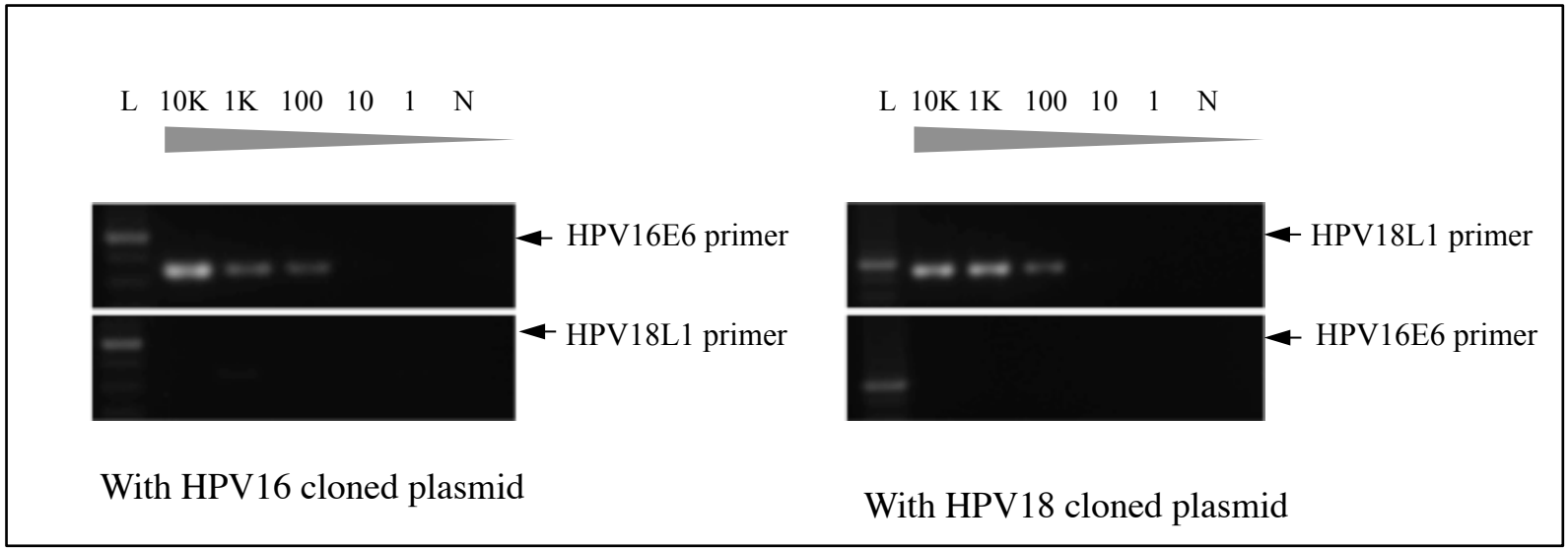
Supplementary Figure 1: Postive and negative control cell lines wers used as positive and negative controls for HPV16 (A,B) and HPV18 (C,D) PCR.



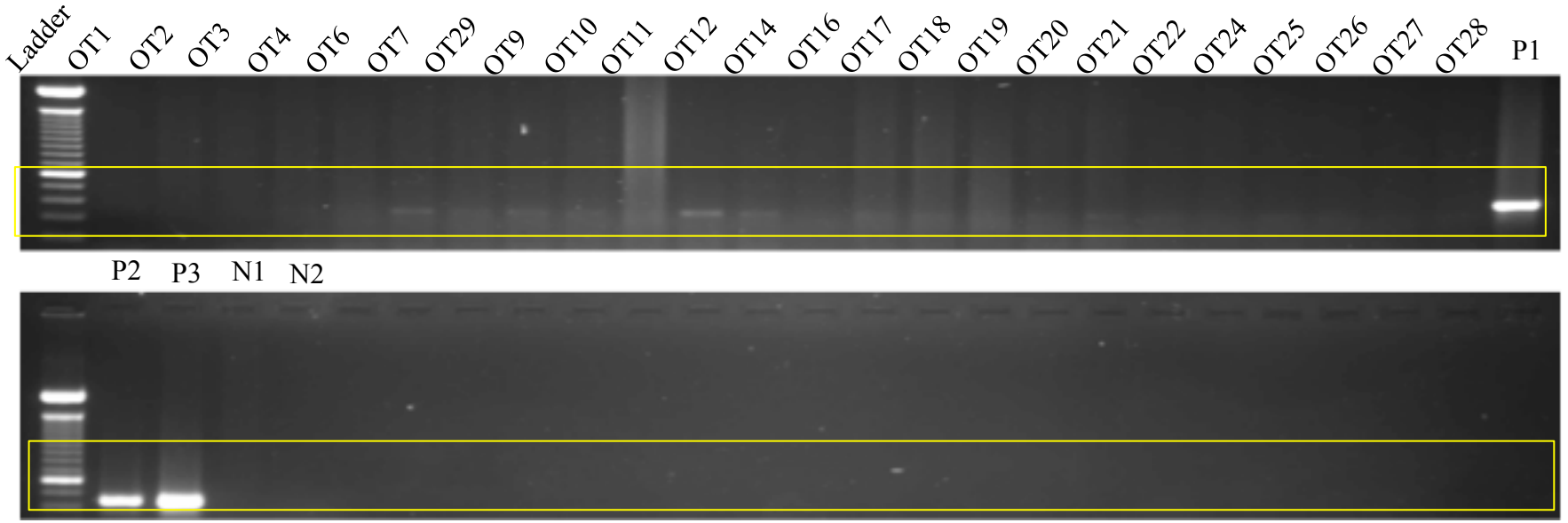
Supplementary Figure 2: Representative image for p16 immunohistochemical staining (IHC) in –ve OSCC +ve OSCC and cervical cancer (+ve control) samples.



Supplementary Figure 3: Efficient amplification of serially diluted HPV16/18 cloned plasmids.

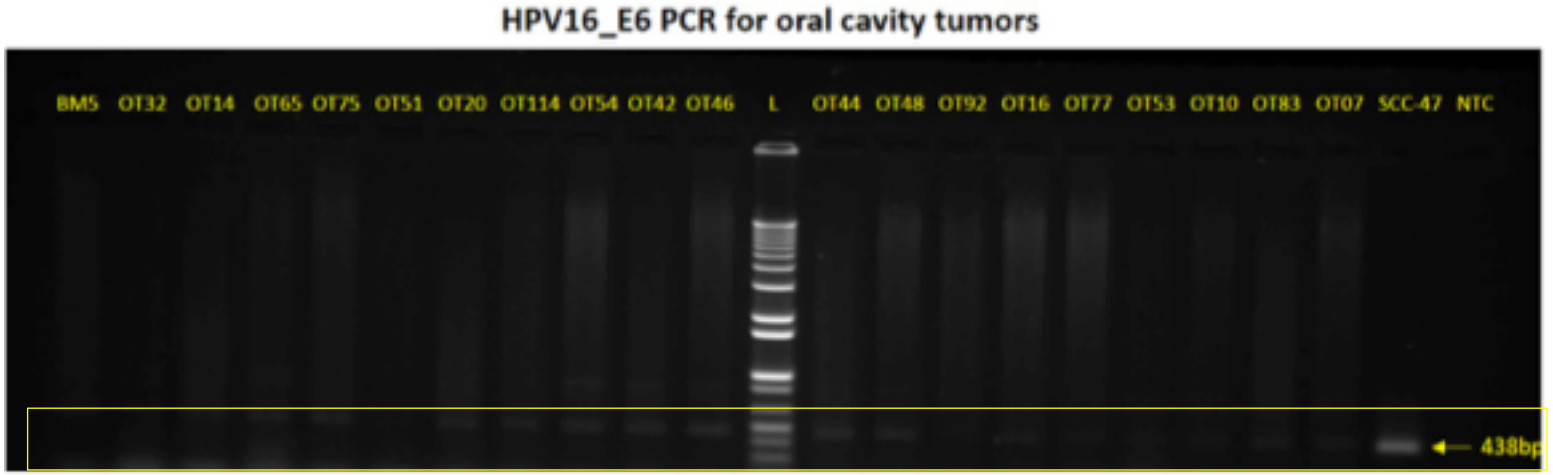


Supplementary Figure 4: PCR with OSCC tumor DNA.

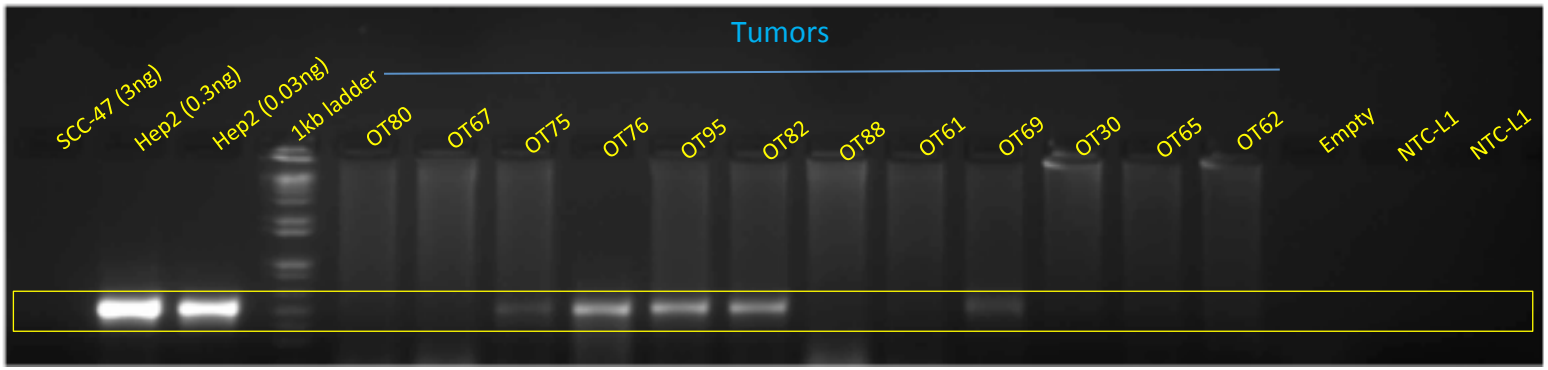


P1-positive control cervical DNA sample1
P2-positive control cervical DNA sample2
P3-UMSCC47 DNA (HPV16 positive cell line)
N1-UPCI:SCC029B DNA (300ng)
N2-No Template Control

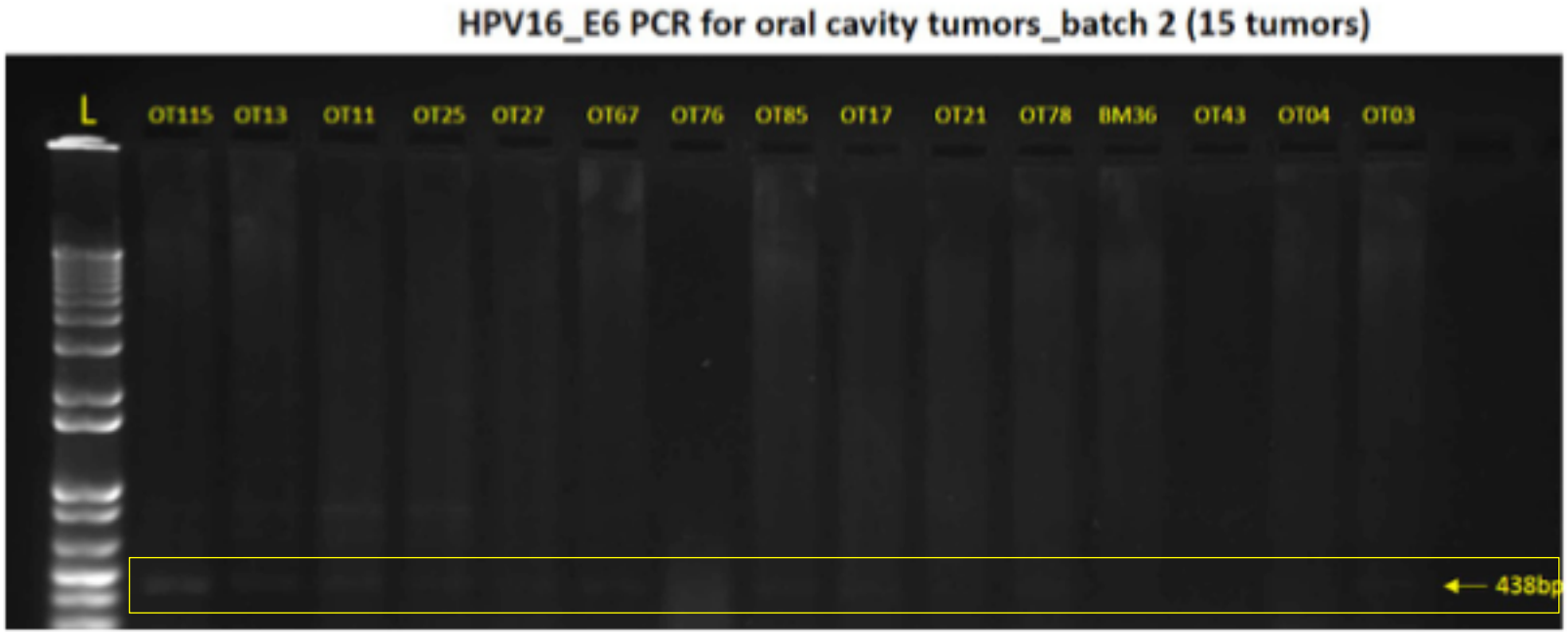
Supplementary Figure 4, contd.: PCR with OSCC tumor DNA



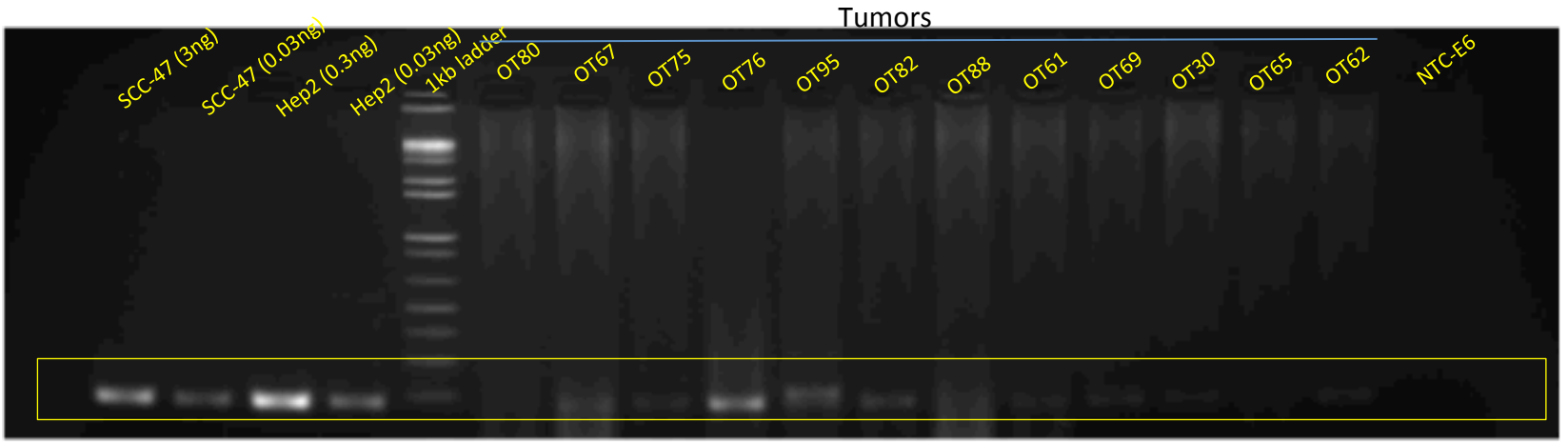
HPV18L1 PCR for oral tumors (using cell lines as p-ve & +ve controls)



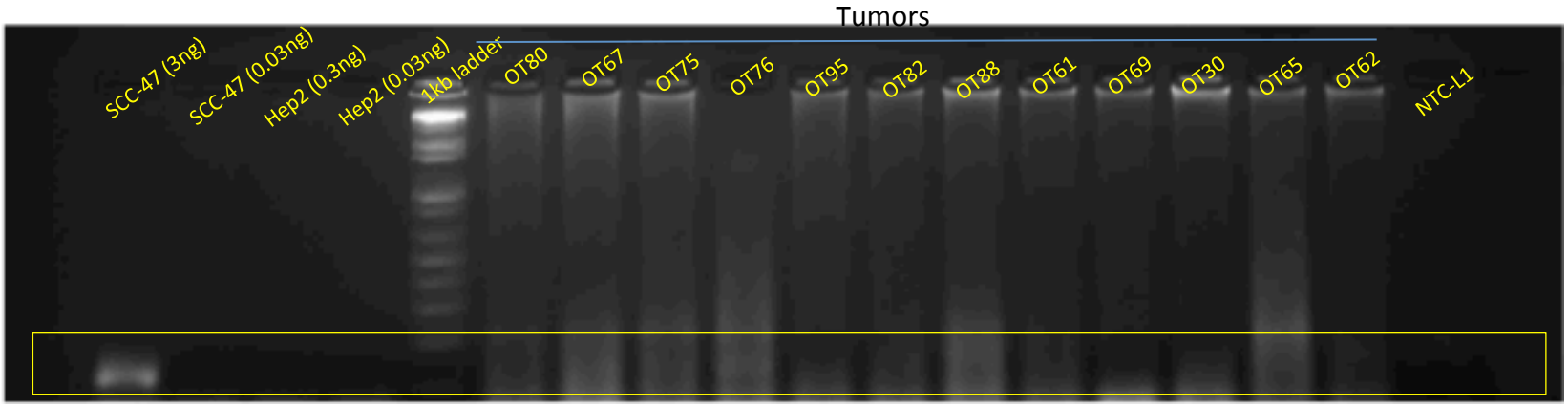
Supplementary Figure 4, contd.: PCR with OSCC tumor DNA



Supplementary Figure 4, contd.: PCR with OSCC tumor DNA

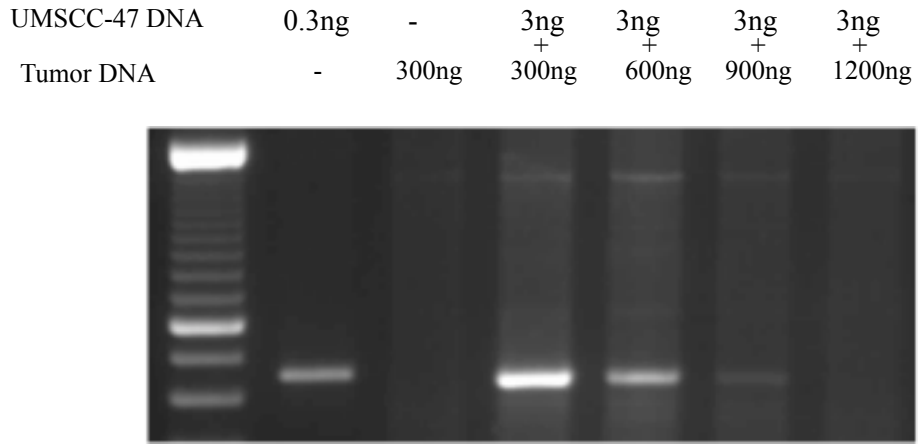


HPV-Cp1-2 PCR oral tumors & using cell lines (positive control)



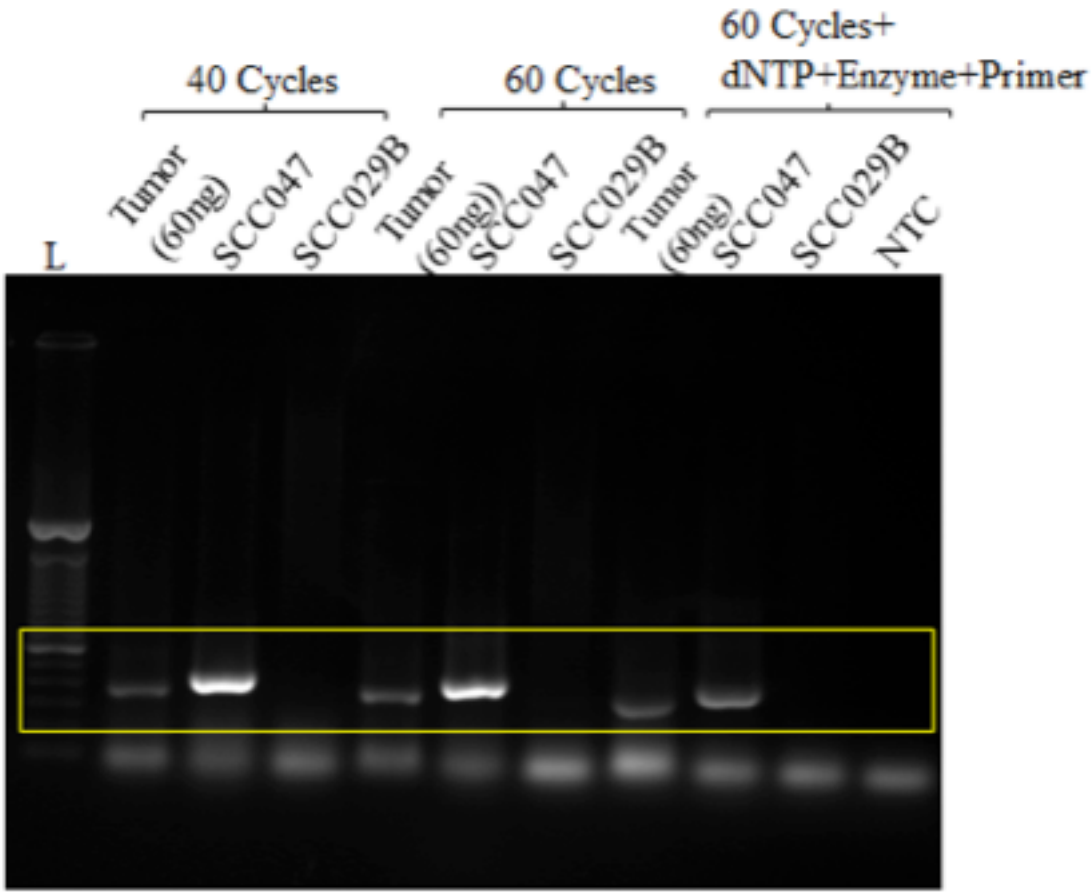
HPV16L1 PCR oral tumors & using cell lines (positive control)

Supplementary Figure 5: Inhibition of amplification reactions at high concentration of tumor genomic DNA with positive cell line spike-in experiment.

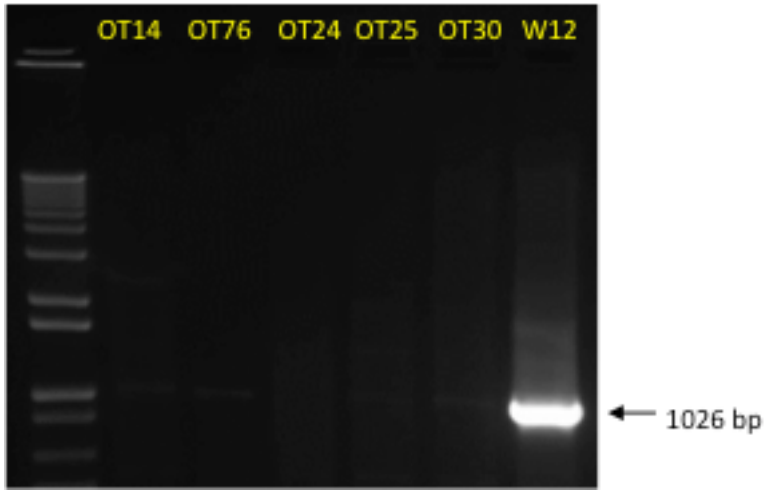


PGMY09-11 PCR using tumor DNA spiked with HPV positive UMSCC-47 DNA

Supplementary Figure 6: An increase in amplification cycles did not yield better results.

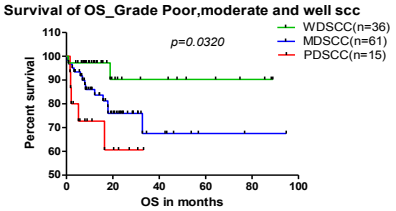


Supplementary Figure 7: HPV E2PCR to test viral integration into host genomes.

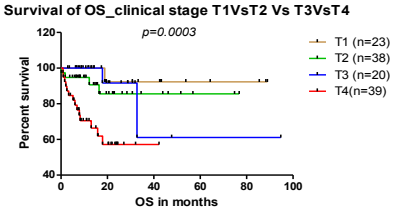


Supplementary Figure 8: KM survival analysis with tumor attributes. With tumor differentiation (A), stage (B-D), habits (E-H), age (I), and nodal status (J).

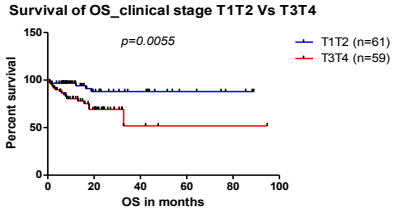
A



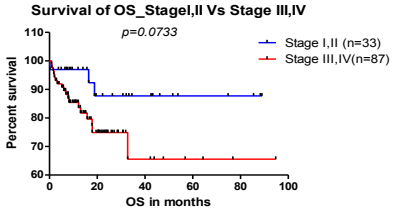
B



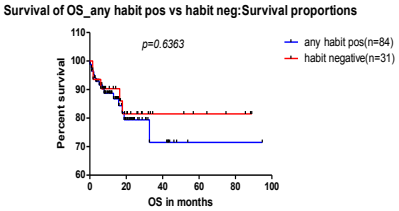
C



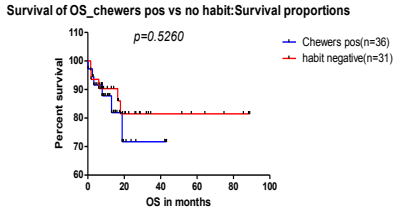
D



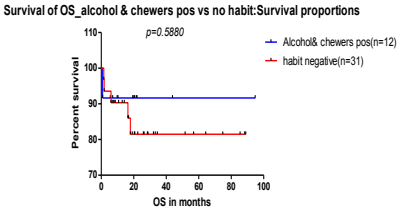
E



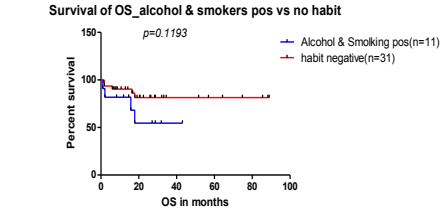
F



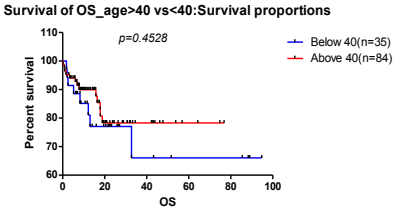
G



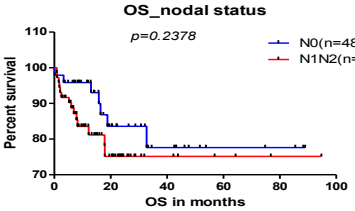
H



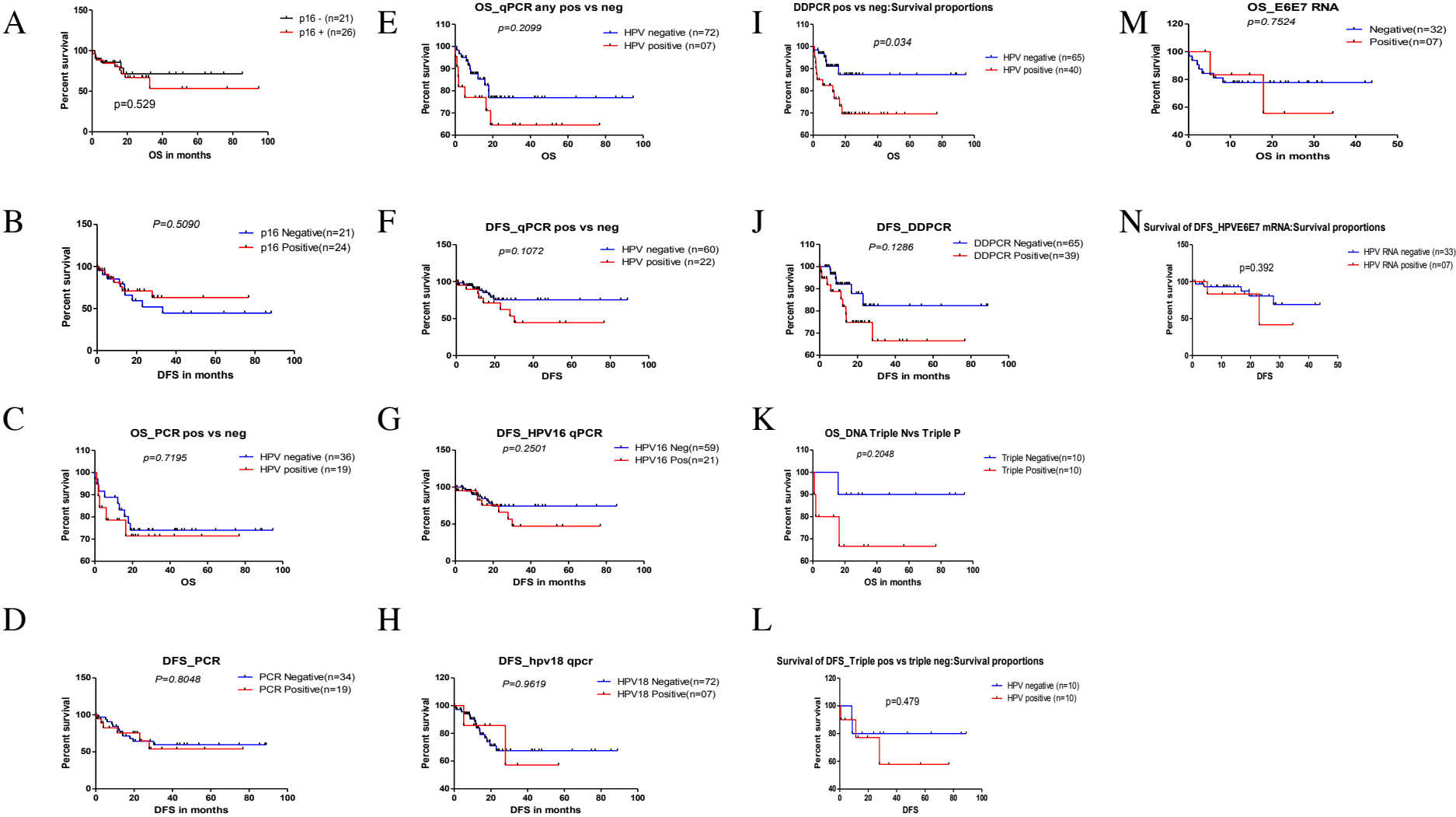
I



J

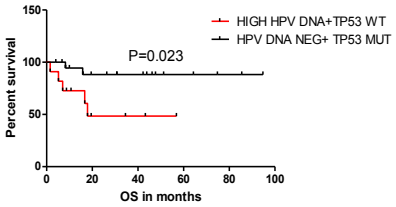


Supplementary Figure 9: KM survival analysis with p16 expression (A,B), HPV DNA as measured by PCR (C,D), qPCR (E-H), ddPCR(I,J), positive in 3/3 DNA-based method (K,L), and E6/E7 mRNA (M,N).

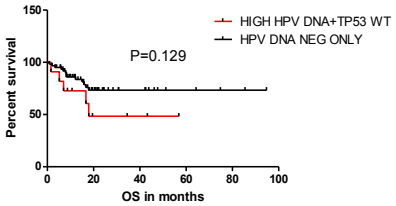


Supplementary Figure 10: KM survival analysis of tumors in various somatic mutational background (A-F).

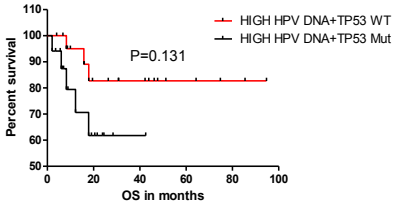
A



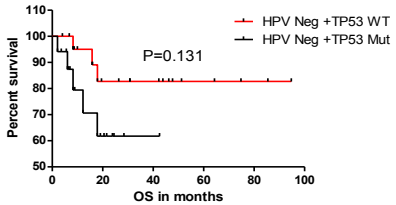
B



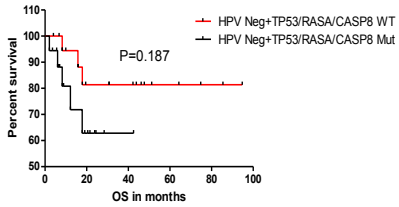
C



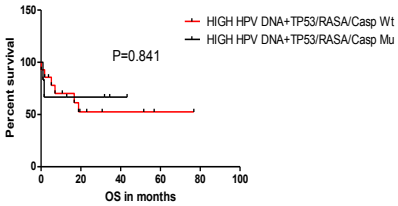
D



E



F



Supplementary Table 1: Patients (n = 153) used in the study.

Characteristics		***Number***
Primary site		
	Buccal mucosa	41
	Oral tongue	112
Gender		
	Male	114
	Female	39
Age		
	>40	112
	<=40	40
	NA	1
Risk habbits		
	Alcohol	4
	Chewing	43
	smoking	6
	Alcohol+ chewing	14
	smoking + alcohol	16
	smoking + chewing	9
	smoking + alcohol+ chewing	10
	No habbits	44
	NA	7
Tumor clasification/stage		
	I+II	43
	III+IV	109
	NA	1
Differentiation		
	Well	48
	Moderate	73
	Poor	20
	NA	12
Abbreviations: NA = Not Available		

Supplementary Table 2: Primer and probe sequences along with amolicon size and the conditions of amplification reactions used for nucleic acid based detection.

Parameter	Assay	Primer	Sequence	Domain	Region (bp)	Amplicon Size (bp)	PCR Conditions	Reference if any
	PCR	HPV16L1		L1	6030 - 6180	151	94°C : 3 min	Pool of 11F & 9 R primers form Gravitt et al., J Clin Microbiol, 2000
							94°C : 60 sec	
			5' TGC TAG TGC TTA TGC AGC AA 3'				55°C : 60 sec	
			3'ATT TAC TGC AAC ATT GGT AC 5'				72°C : 60 sec	
							40 cycles	
							72°C : 2 min & 4°C hold	
		GP5+/6+		L1	6624- 6746	150	94°C : 5 min	
							94°C : 60 sec	
			5' TTT GTT ACT GTG GTA GAT AC 3'				57.8 °C : 60 sec	
			3' GAA AAA TAA ACT GTA AAT CA 5'				72°C : 30 sec	
							40 cycles	
							72°C : 7 min & 4°C hold	
		MY09/11		L1	6602 - 7034	450	94°C : 5 min	
							94°C : 60 sec	
			3' GCM CAG GGW CAT AAY AAT GG 5'				57.8 °C : 60 sec	
			5' CGT CCM ARR GGA WAC TGA TC 3'				72°C : 60 sec	
							40 cycles	
							72°C : 7 min & 4°C hold	
		CP I/II		E1	1777 - 1942	188	94°C : 5 min	
							94°C : 60 sec	
			5' TTA TCW TAT GCC CAY TGT ACC AT 3'				61.7°C:60sec	
			3' ATG TTA ATW SAG CCW CCA AAA TT 5'				72°C : 30 sec	
							40 cycles	
							72°C : 7 min & 4°C hold	
		PGMY09/11	Pool of 11F & 9 R primers form Gravitt et al., J Clin Microbiol, 2000	L1	6602 - 7034	450	94°C : 5 min	
							94°C : 60 sec	
							57.8 °C : 60 sec	
							72°C : 60 sec	

							40 cycles	
							72°C : 7 min & 4°C hold	
		HPV16E6 PCR primer		E6	119-556		94°C : 3 min	Newly designed
							94°C : 30 sec	
			5’ CAG GAG CGA CCC AGA AAG TT 3’			438	53°C : 30 sec	
			3 CAG CTG GGT TTC TCT ACG TGT 5’				72°C : 30 sec	
							40 cycles	
DNA							72°C : 2 min & 4°C hold	
		HPV18L1 PCR primer		L1	6141-6676	536	94°C : 3 min	
							94°C : 40 sec	
			5’ TCG CGT CCT TTA TCA CAG GGC GA 3’				55°C : 40 sec	
			3’ TGC CCA GGT ACA GGA GAC TGT G 5’				72°C : 30 sec	
							40 cycles	
							72°C : 2 min & 4°C hold	
	qPCR	HPV16E6 cloning primer		E6	119 - 556	438	as described above	
			5’ CAG GAG CGA CCC AGA AAG TT 3’					
			3 CAG CTG GGT TTC TCT ACG TGT 5’					
		HPV16E6 qPCR		E6	150 - 256	107	95°C : 3 min	
			5’ GCA CAG AGC TGC AAA CAA CT 3’				95°C : 30 sec	
			3’ GCA TAA ATC CCG AAA AGC AA 5’				55°C : 30 sec	
			probe-ATTAGAATGTGTGTACTGCAAGCA-FAM-BHQ				72°C : 30 sec	
							40 cycles followed with dissociation curve	
		HPV18L1 cloning primer		L1	6141 - 6676	536	as described above	
			5’ TCG CGT CCT TTA TCA CAG GGC GA 3’					
			3’ TGC CCA GGT ACA GGA GAC TGT G 5’					

		HPV18L1 qPCR		L1	6416 - 6506	91	95°C : 3 mints	
			5’ TGA CAC TGT GCC TCA ATC CT 3’				95°C : 30 sec	
			3’ AGA GCC ACT TGG AGA GGG AG 5’				60°C : 30 sec	
			Probe-TGCCTGCTTCACCTGGCAGC-VIC-BHQ				72°C : 30 sec	
							40 cycles followed with dissociation curve	
	ddPCR	HPV16E6 DD-PCR		E6	417 - 554	138	95°C : 10 min	
			5’ ACT GTC AAA AGC CAC TGT GT 3’				95°C : 15 sec	
			3’ GCT GGG TTT CTC TAC GTG TT 5’				55°C : 20 sec	
			Probe-AGGGGTCGGTGGACCGGTCGATGT-FAM-BHQ				40 cycles	
							95°C : 10 min	
RNA	**E6**	HPV16_E6_RTPCR using SYBR chemistry					95°C : 3 mints	
			GCACCAAAAGAGAACTGCAATGTT		85-108	152	95°C : 3 sec	Catani et al, 2009; doi: 10.1128/JCM.01733-09
			AGTCATATACCTCACGTCGCAGTA		197-236		60°C : 30 sec	
							40 cycles followed with dissociation curve	
		HPV18_E6_RTPCR using SYBR chemistry	CTATAGAGGCCAGTGCCATTCG		503-524	79	Same as above	Catani et al, 2009; doi: 10.1128/JCM.01733-12
			TTATACTTGTGTTTCTCTGCGTCG		558-581			
	E7	HPV16_E7_RTPCR using SYBR chemistry						
			CAAGTGTGACTCTACGCTTCGG		738-759	81	Same as above	Catani et al, 2009; doi: 10.1128/JCM.01733-18
			GTGGCCCATTAACAGGTCTTCCAA		796-818			
		HPV18_E7_RTPCR using SYBR chemistry						
			TAATCATCAACATTTACCAGCCCG		721-744	113	Same as above	Catani et al, 2009; doi: 10.1128/JCM.01733-22
			CGTCTGCTGAGCTTTCTACTACTA		810-833			
	GAPDH	GAPDH	CTGCACCACCAACTGCTTAG			105	Same as above	Bustin et al, 2011; doi:10.3409/fb62_1.73
			TTCTGGGTGGCAGTGATG					
Integration Assay	**E6 PCR**	HPV16E6	AAGGGCGTAACCGAAATCGGT			209	as described earlier	Gallo et al, Clin Pathol 2003;56:532–536
			CATATACCTCACGTCGCAG					
	W1-W2 regio	E2 PCR	ATGAAAATGATAGTACAGAC		2811-3837	1026	as described earlier	Sengupta et al, Gynecologic Oncology 100 (2006) 372 – 378 & Gallo et al, Clin Pathol 2003;56:532–536
			CCAGTAGACACTGTAATAG					

Supplementary Table 3: *p*-values from unpaired t-tests measuring significance in differences between differential methylation in 9 HPV associated genes between HPV +ve and HPV -ve group. Group 1: when high-copy and/or HPV E6/E7 RNA is taken into consideration to define HPV positivity, and Group 2: when HPV DNA only, irrespective of copy number, is taken into consideration to define HPV positivity.

Genes	Group 1 HPV +ve vs HPV -ve	Group 2 HPV +ve vs HPV -ve
FERMT3	< 0.00001	0.0346
GIT2	< 0.00001	0.1052
HK3	< 0.00001	0.0574
PRKCZ	< 0.00001	0.052
ZCCHC8	< 0.00001	0.0504
IRF5	< 0.00001	0.083
IFFO1	< 0.00001	0.0608
ARID3A	< 0.00001	0.0654
HOXA2	0.0074	0.1788