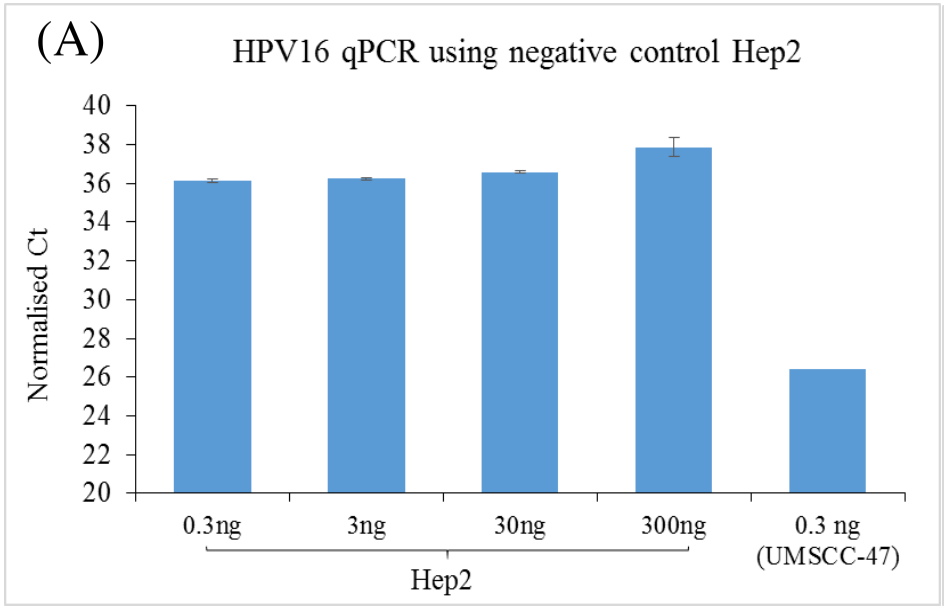
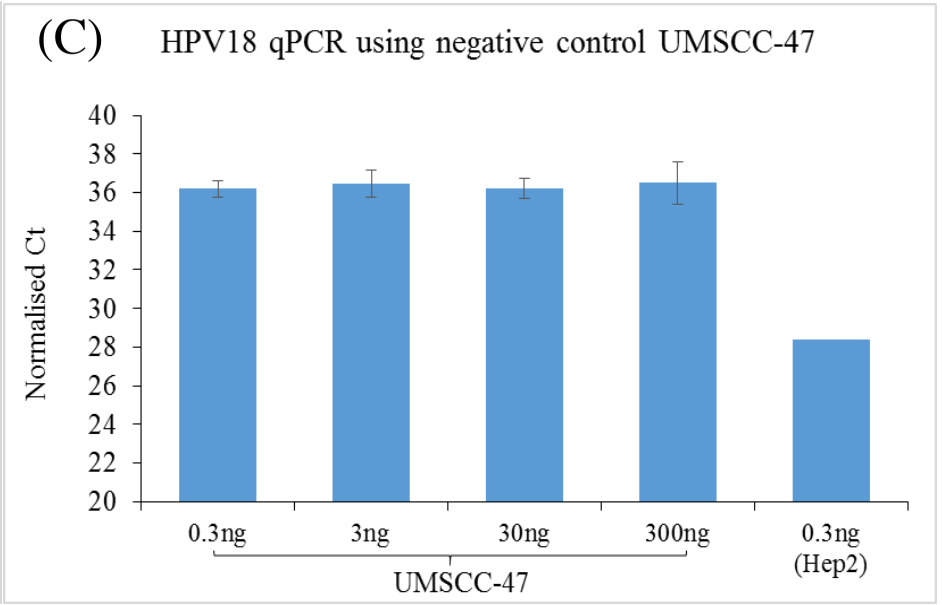
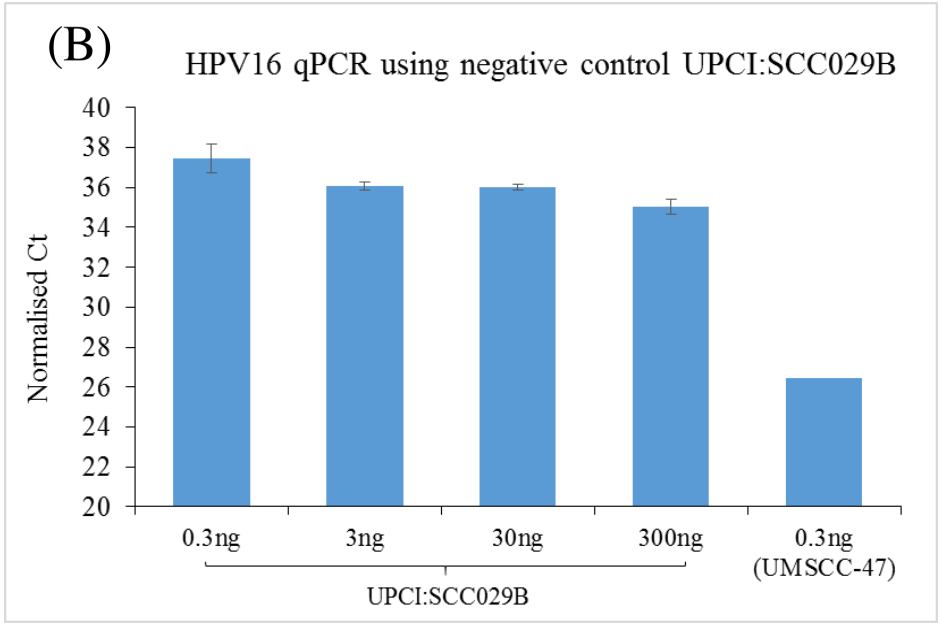
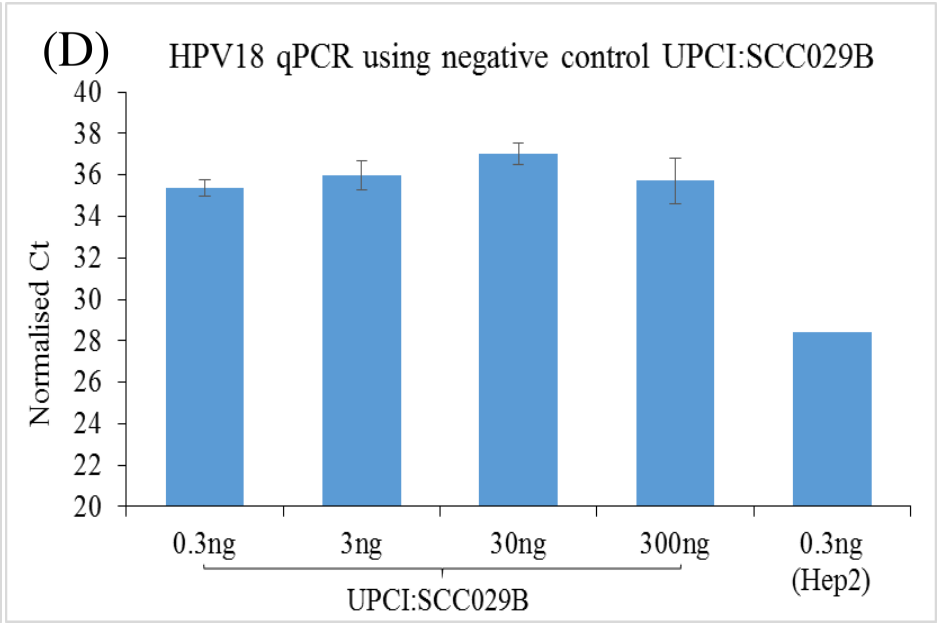
Supplementary Figure 1: Increasing amount of genomic DNA from cell lines used as positive or negative controls for HPV16 (A, B) and HPV18 (C, D) PCR.

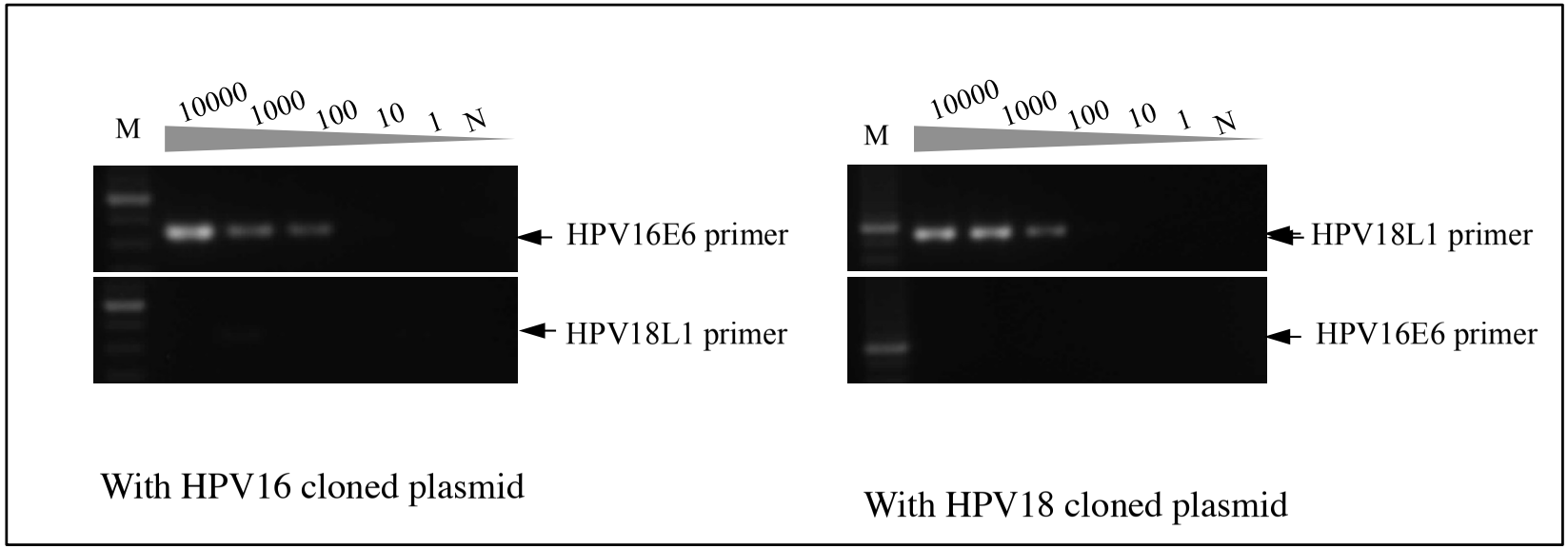




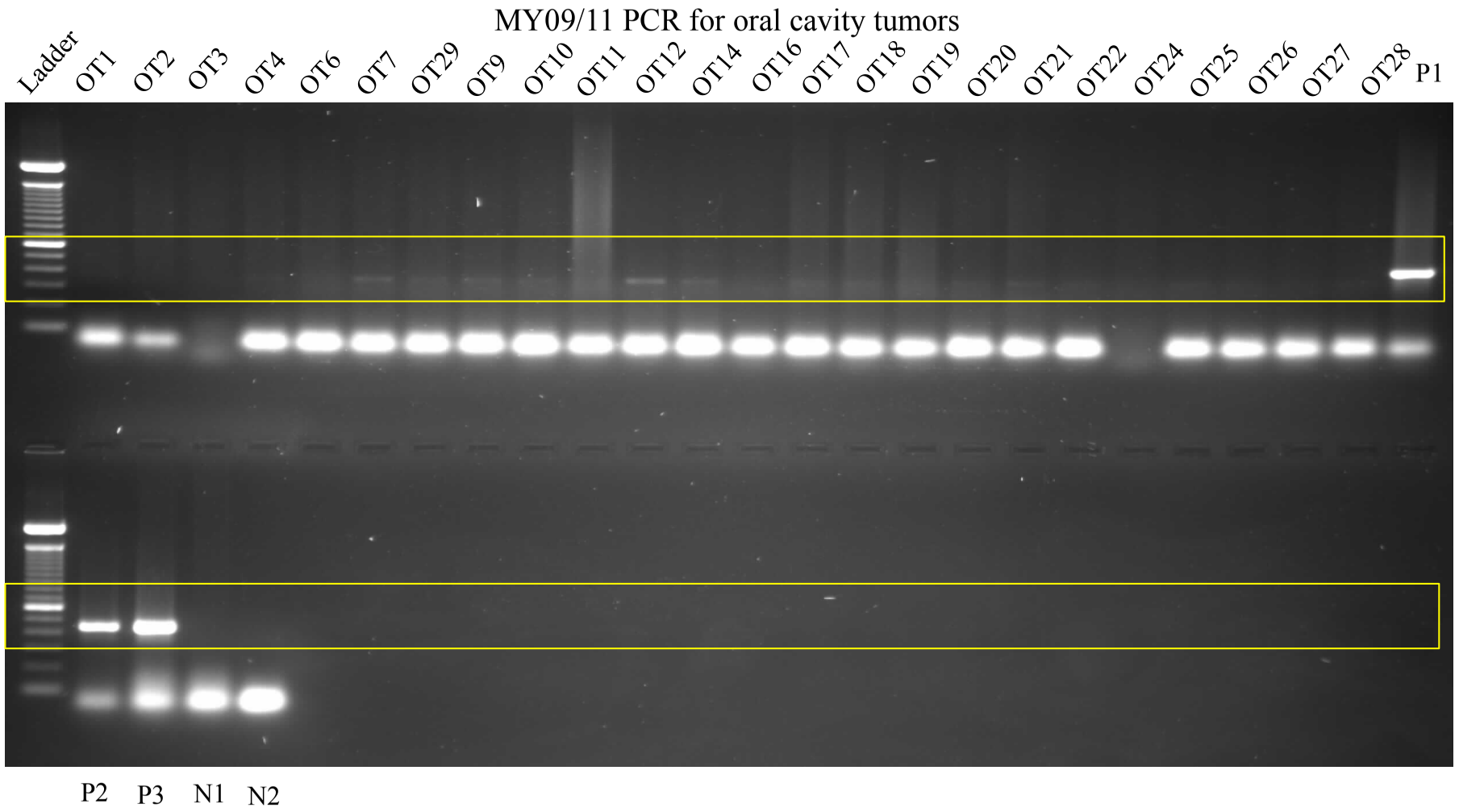




Supplementary Figure 2: Amplification efficiency of HPV16E6 and HPV18L1 primers measured by PCR amplification of serially diluted HPV16/18 cloned plasmid copies.

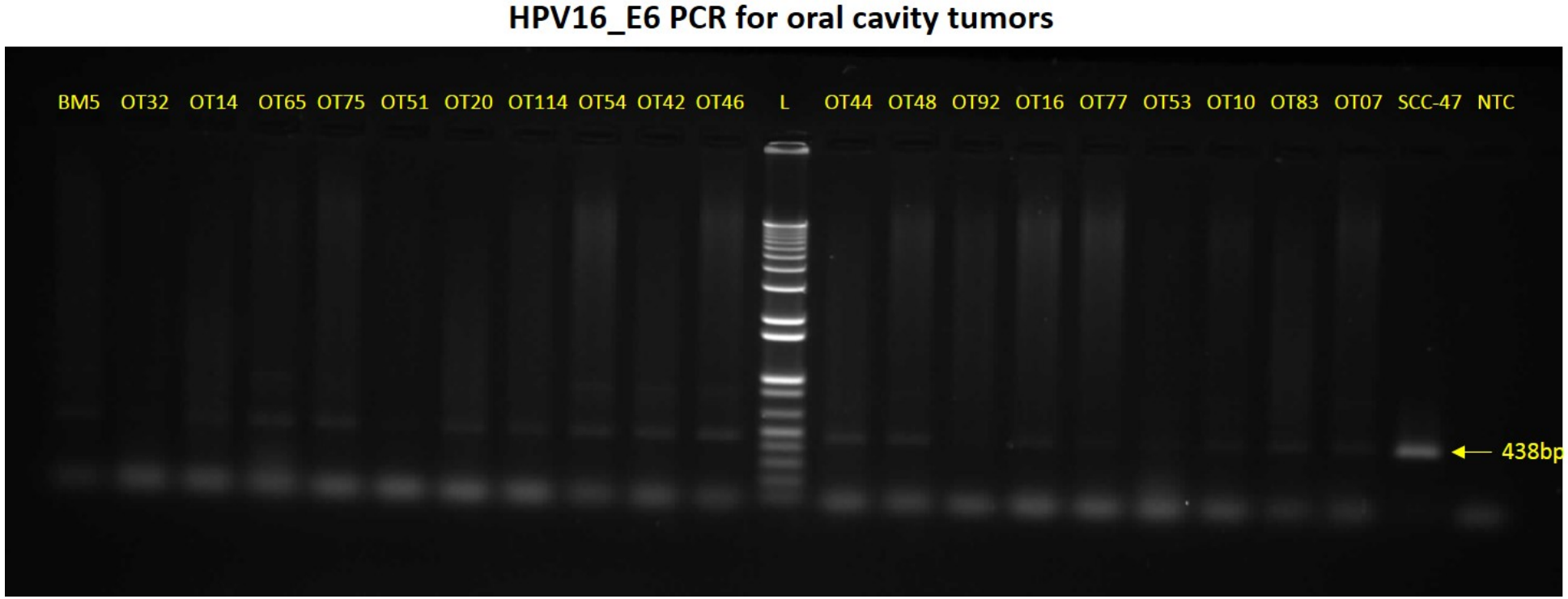


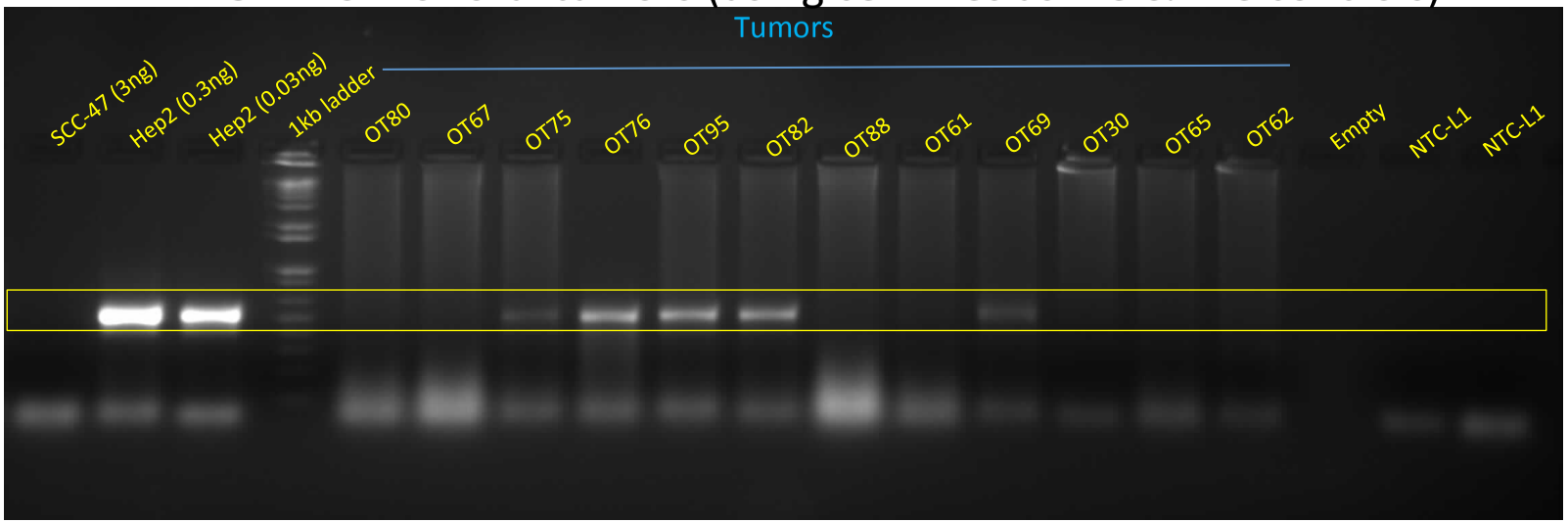
Supplementary Figure 3: HPV PCR performed using different sets of consensus/type specific primers with OSCC tumor DNA. OT: oral tongue, BM: buccal mucosa.



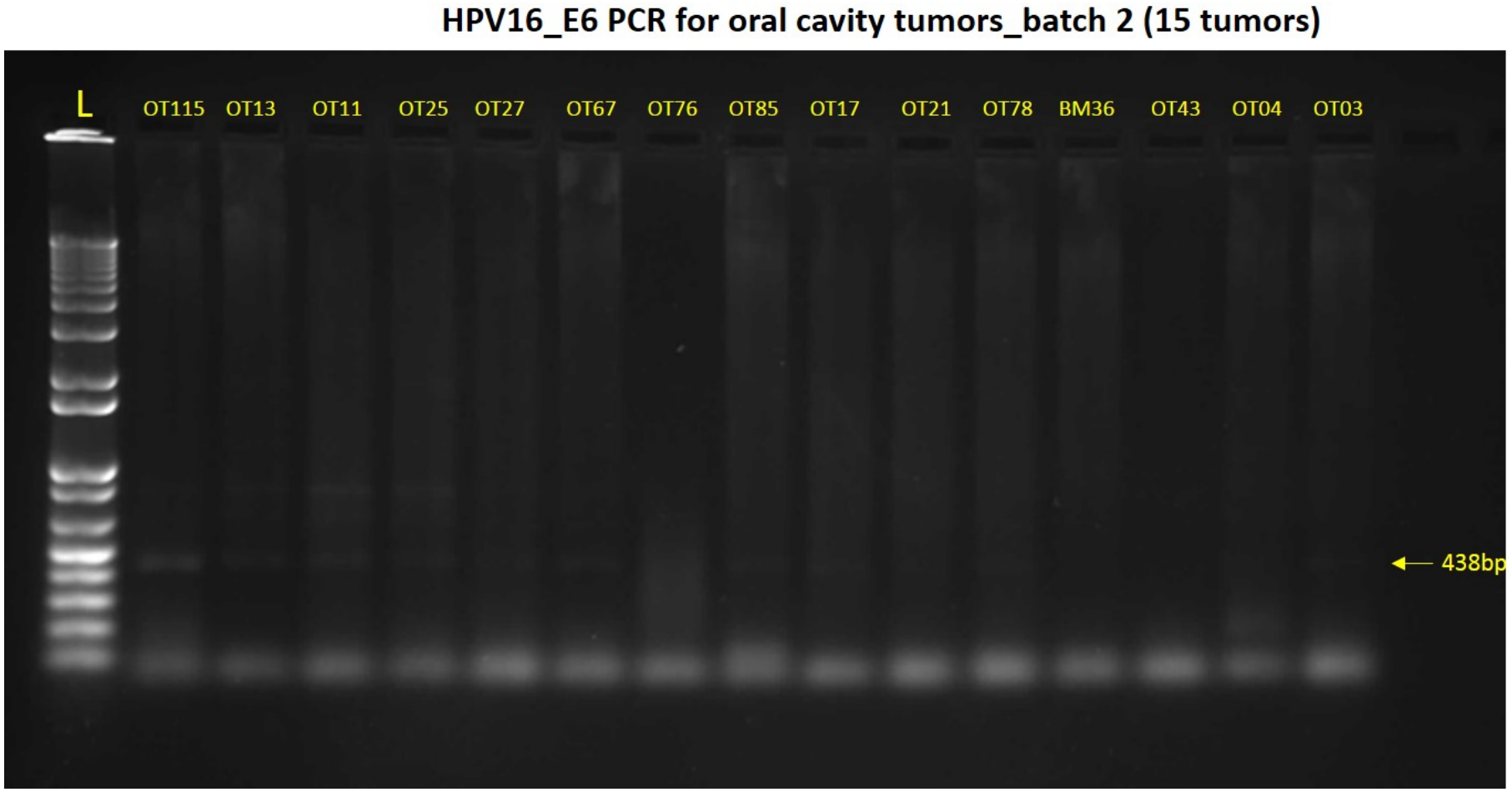
P1-positive control cervical DNA sample1
P2-positive control cervical DNA sample2
P3-UMSCC47 DNA (HPV16 positive cell line)
N1-UPCI:SCC029B DNA (300ng)
N2/NTC-No Template Control

Supplementary Figure 3, contd.

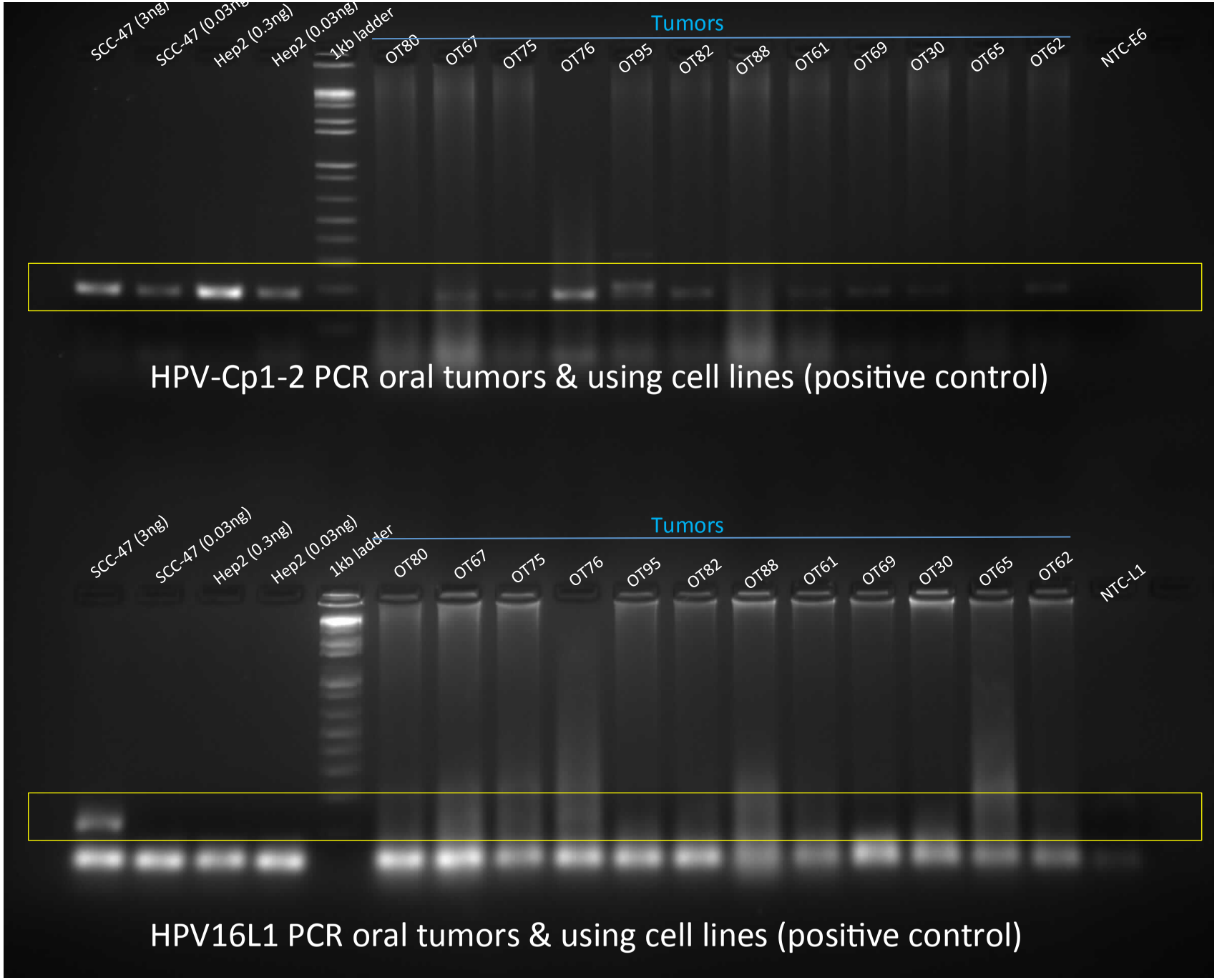




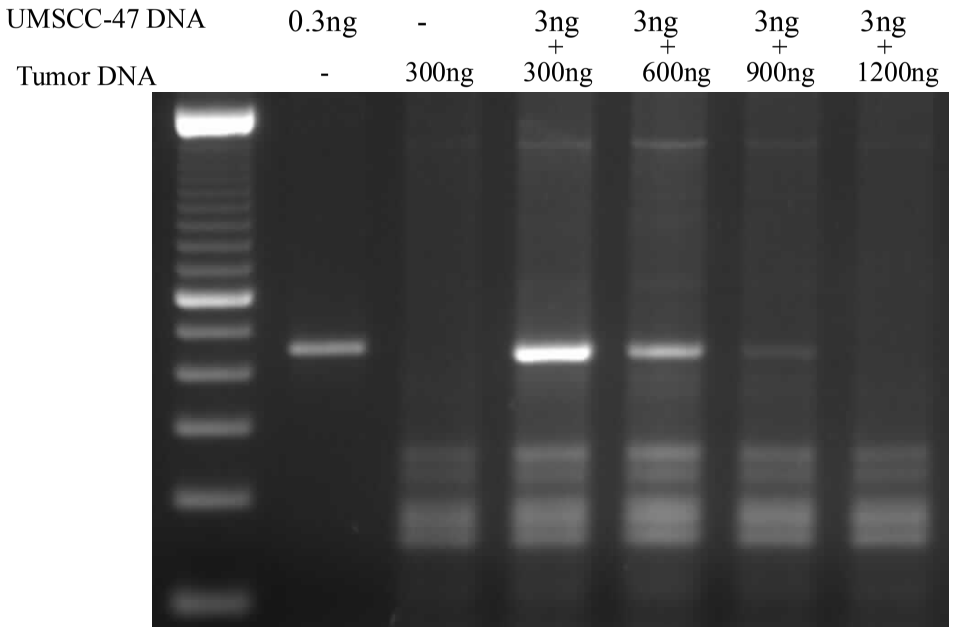
Supplementary Figure 3, contd.



Supplementary Figure 3, contd.

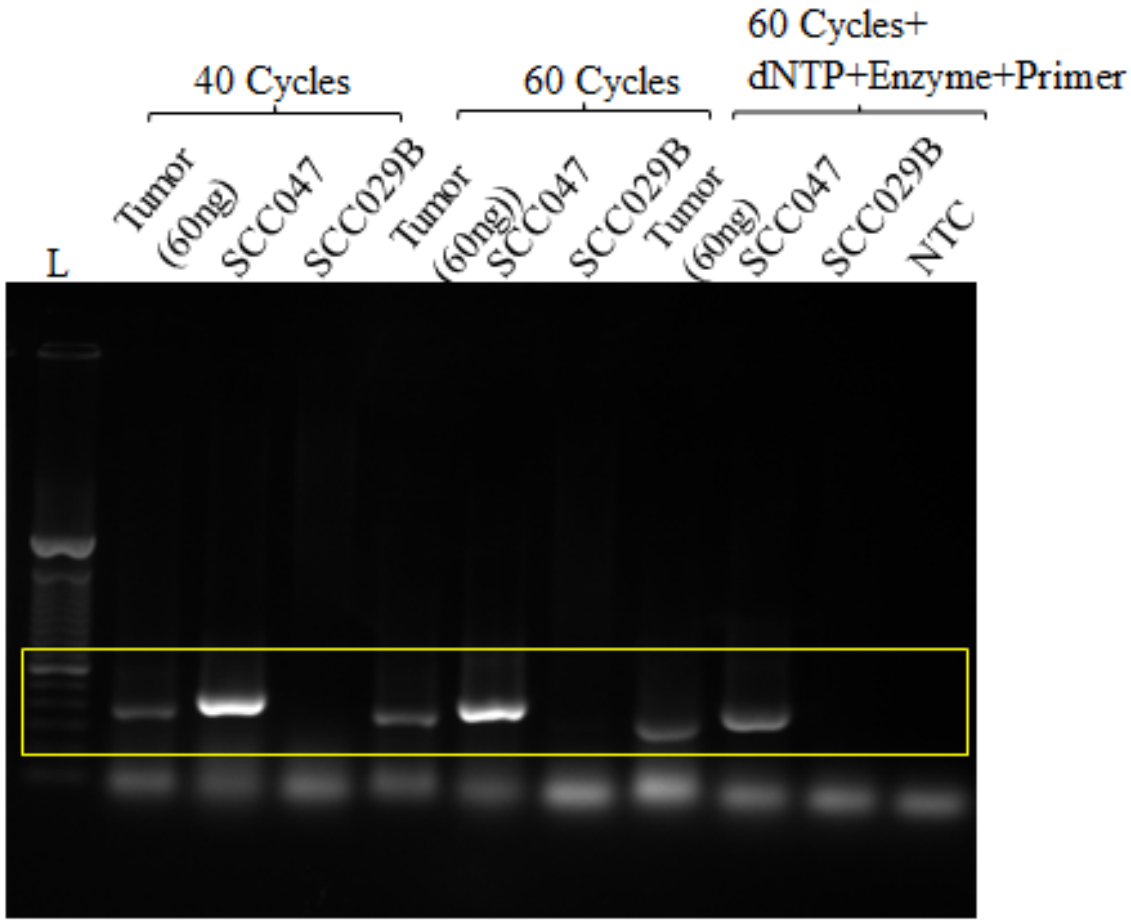


Supplementary Figure 4: Inhibition of amplification reactions in HPV detection in PCR at high concentration of tumor genomic DNA with spike-in experiment using UM-SCC47 cell line.

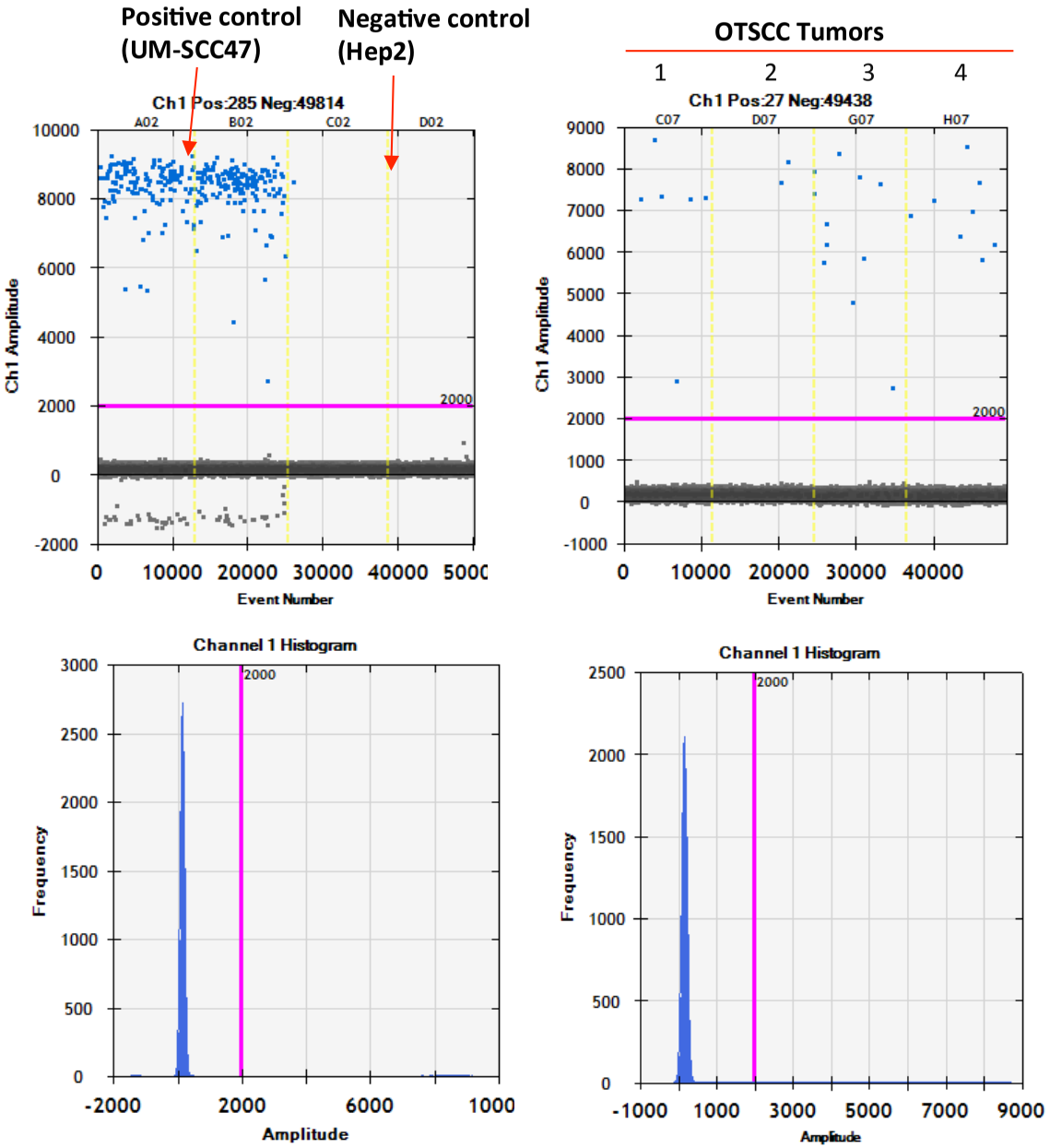


PGMY09-11 PCR using tumor DNA spiked with HPV positive UMSCC-47 DNA

Supplementary Figure 5: The effect of amplification cycle on PCR. The genomic DNA used for positive control (UM-SCC-47) and negative control cell lines were 6, 3.0ng and 300ng respectively.

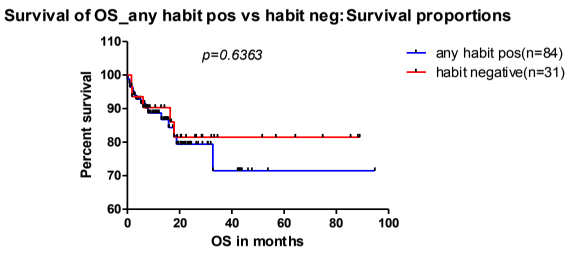


Supplementary Figure 6: Positive and negative cell line DNA used for threashold in ddPCR experiment.

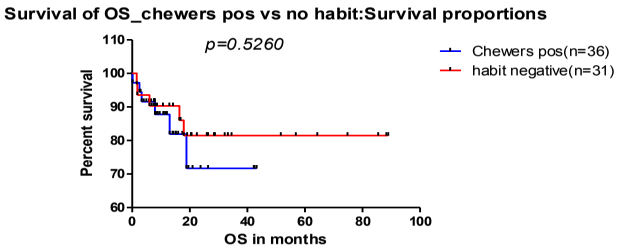


Supplementary Figure 7: KM survival analysis with tumor with habits (A-D), age (E), and nodal status (F).

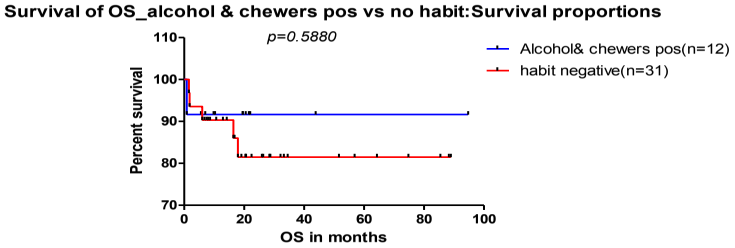
A



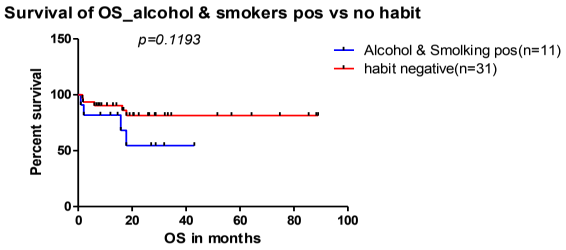
B



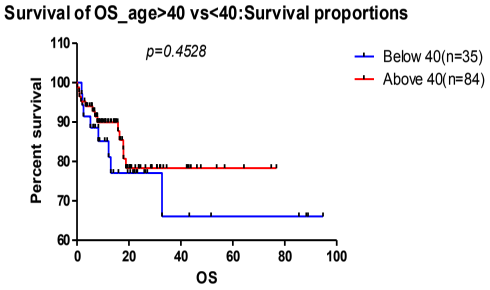
C



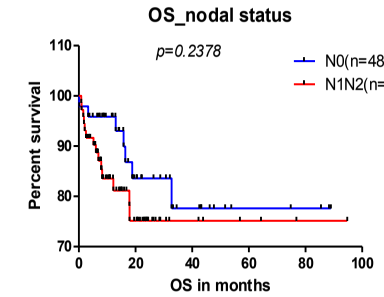
D



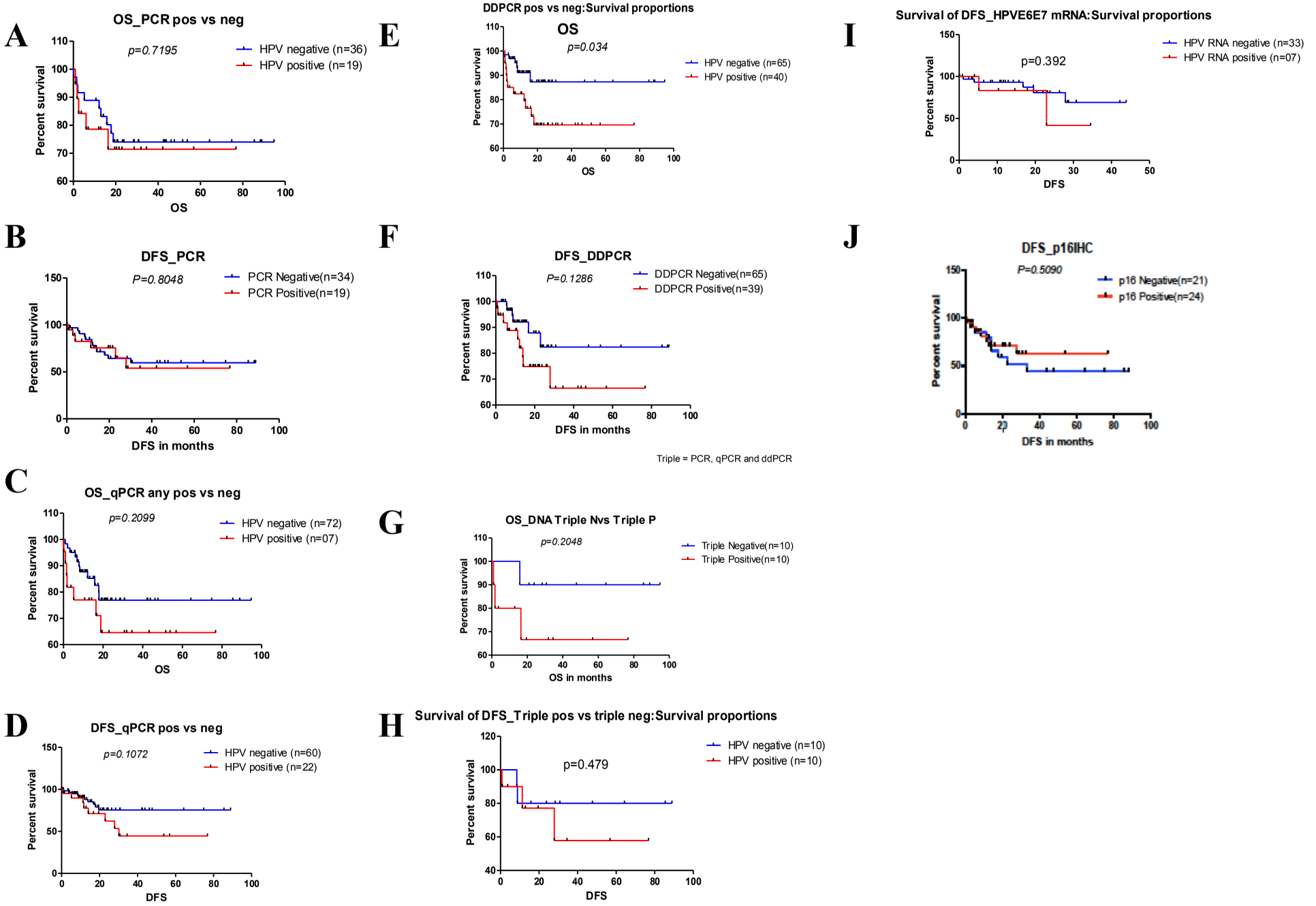
E



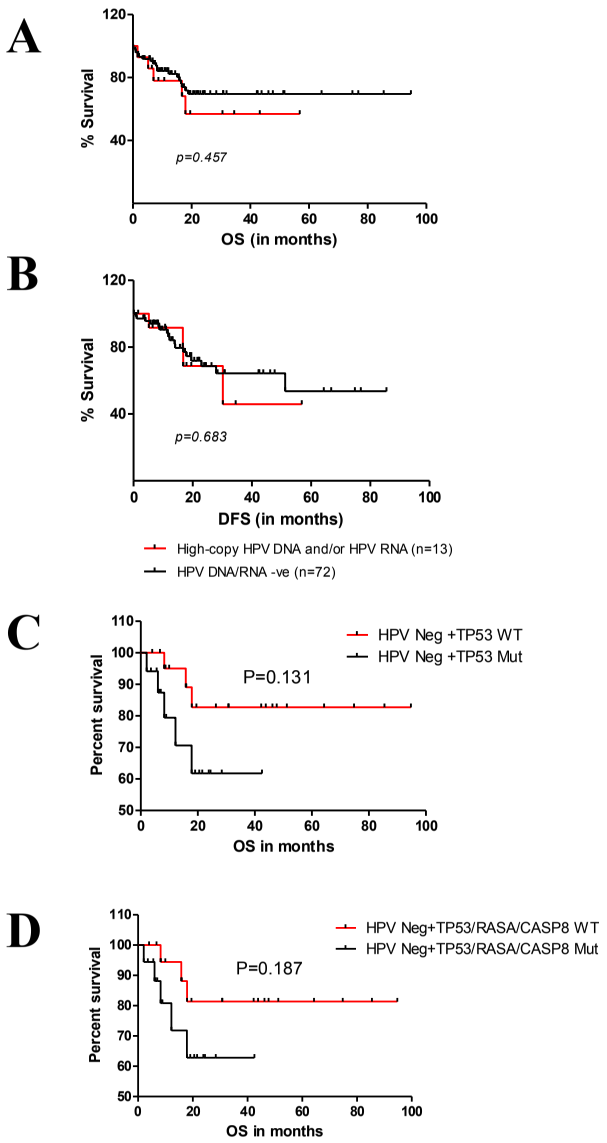
F



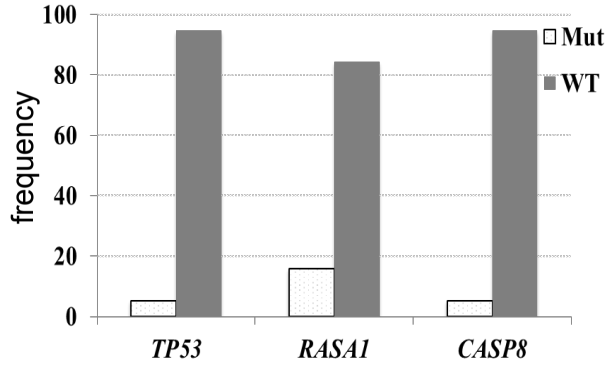
Supplementary Figure 8: KM survival analysis with HPV DNA (A-H), and RNA (I,J).



Supplementary Figure 9: KM survival analysis with tumors with high copy DNA and/or HPV RNA (A, B) and HPV –ve tumors with mutations in significant genes (C, D)..



Supplementary Figure 10: Mutational frequency in tumors with mutations in three commonly mutated genes.



Supplementary Table 1: Primer and probe sequences used in the study along with amolicon size and the conditions of amplification reactions. All the primers were desigened using using NC_001526.4 and NC_001357.1 sequences from NCBI for HPV16 and HPV18 respectively.

Parameter	Assay	Primer	Sequence	Domain	Region (bp)	Amplicon Size (bp)	PCR Conditions	Reference if any
DNA	**PCR**	HPV16L1		L1	6030 - 6180	151	94°C : 3 min	Pool of 11F & 9 R primers form Gravitt et al., J Clin Microbiol, 2000; Karlsen et al J of clinical micri0biology, 1996
							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	
			5'-CGT CCM ARR GGA WAC TGA TC-3'				57.8 °C : 60 sec	
			5' GCM CAG GGW CAT AAY AAT GG 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 used for PCR
					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	
					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	Used for cloning HPV16E6 region in PUC19 plasmid.
			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	Used for cloning HPV18L1 region in PUC19 plasmid.
			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	E6	85-108	152	95°C : 3 sec	Cattani et al, 2009; doi: 10.1128/JCM.01733-08
			AGTCATATACCTCACGTCGCAGTA		197-236		60°C : 30 sec	
							40 cycles followed with dissociation curve	
		HPV18_E6_RTPCR using SYBR chemistry	CTATAGAGGCCAGTGCCATTCG	E6	503-524	79	Same as above	Cattani et al, 2009; doi: 10.1128/JCM.01733-08
			TTATACTTGTGTTTCTCTGCGTCG		558-581			
	E7	HPV16_E7_RTPCR using SYBR chemistry						
			CAAGTGTGACTCTACGCTTCGG	E7	738-759	81	Same as above	Cattani et al, 2009; doi: 10.1128/JCM.01733-08
			GTGGCCCATTAACAGGTCTTCCAA		796-818			
		HPV18_E7_RTPCR using SYBR chemistry						
			TAATCATCAACATTTACCAGCCCG	E7	721-744	113	Same as above	Cattani et al, 2009; doi: 10.1128/JCM.01733-08
			CGTCTGCTGAGCTTTCTACTACTA		810-833			
	GAPDH	GAPDH	CTGCACCACCAACTGCTTAG	NA	7537-7641	105	Same as above	Szostek et al, 2014; doi:10.3409/fb62_1.73
			TTCTGGGTGGCAGTGATG					
Sanger sequencing for mutation study-			As described in Krishan et al. (2015). doi: 10.12688/f1000research.7302.1					

All the primers were aligned/desigened using using NC_001526.4 and NC_001357.1 sequences from NCBI for HPV16 & HPV18 respectively.

Supplementary Table 2: *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

Supplementary Table 3: Literature survey on HPV studies in oral cavity tumors.

Reference	Cohort	Subsite	Patient No.(n)	Method/Marker of HPV detection									HPV DNA Prevelence	HPV subtype prevalence	p16 Prevalence	HPV DNA linked with ourcome	p16 linked with ourcome	
				HPV-DNA by PCR/qPCR/RFLP/sequencing	HPV Genotyping INNO-LiPA	PCR-Mass Array	DNA-PCR-Dot Blot	DNA by ISH	HPV-RNA by qRT-PCR (E2/E6/E7)	p16 IHC	E6/E7 IHC	E6/E7 antibody-ELISA						Comments
Huang et al, 2014; PMID:25097016	Taiwan	Oral Cavity	312	●	○	○	○	○	○	○	○	○	16.6	HPV16	NA	high HPV 16/18 E7 viral load identifies a small subgroup of patients at high-risk of 5-year DM	NA	0
Lee et al,2012; PMID:22808258	Taiwan	Oral Cavity	333	●	○	○	●	○	○	○	○	○	21.3	HPV16	NA	Oral Cavity Cancer Patients Is Related to an Increased Risk of Distant Metastases and Poor	NA	0
Gracia et al, 2014; PMID:24268899	Spain	Oral tongue	64	●	●	○	○	○	○	○	○	○	26.2	HPV56	NA	mortality showed a statistically significant correlation, being higher in HR-HPV patients	NA	0
Lee et al, 2015; PMID:26632712	Taiwan	oral Cavity	1002	○	○	○	●	○	○	○	○	○	19	HPV16	NA	patients and predict 5-year OS; 5-year OS rate of HPV-positive patients was significantly lower	NA	0
Lee et al, 2013; PMID:23669598	Taiwan	Oral Cavity	410	○	○	○	●	○	○	○	○	○	21.2	HPV16	NA	DFS, disease-specific survival and OS in the subgroups of OSCC patients with poor	NA	0
Ringstrom et al, 2002; PMID:12374687	USA	Oral Cavity & others*	41	●	○	○	○	○	○	○	○	○	5	HPV16	NA	habiit shows better clinical outcome than HPV-negative group	NA	1
Smith et al,2008; PMID:17360226	USA	Oral Cavity & others*	170	●	○	○	○	○	○	●	○	○	15	HPV16	25	HR-HPV is positive predictor of outcome	predictor of outcome	1
Smith et al,2010; PMID:19876924	USA	Oral Cavity & others*	21	○	○	○	●	○	○	●	○	●	15.8 e6/e7	NA	NA	positive tumors, distinguishable by E6 and/or E7 antibody status. Differences in antibody status	NA	3
al,2008:PMID:18268127	USA	Oral Cavity & others*	166	●	○	○	●	○	○	○	○	○	16	HPV16	NA	different HRs for each clinical outcome (p53 overexpression=48%); p53/HPV provides a	NA	1
Zhao et al,2009;PMID: 20695077	China	Oral Cavity	52	●	○	○	○	○	○	○	○	○	40.4	HPV16	NA	HPV was significantly correlated with a better survival of OSCC	NA	1
Ramshankar et al, 2014; PMID:25339028	Indian	Oral tongue	167	●	○	○	○	○	○	○	○	○	52	HPV16	15.3	HPV 16 DNA was not significant predictor for DFS and disease outcome.	was found to be a significant	3
Cristine et al, 2014; PMID:PMC4251957	USA	Oral cavity & others*	89/80	○	○	○	○	●	○	●	○	○	14.6 (89)	HPV 16	26.3 (80)	between HPV ISH–positive and –negative patients; Moreover, p16-positive OPSCC have	positive tumors had significantly	3
al,2014;PMID:PMC4208923	Venezuela	Oral Cavity & others*	25	○	●	○	○	○	○	○	○	○	35.4	HPV16	NA	HPV positivity in SCC is mainly associated with high-risk HPV	No correlation	2
Grobe et al, 2013; PMID:23721566	Germany	Oral cavity & others*	222	●	○	○	○	○	○	●	○	○	6.9	NA	NA	recurrence free survival of hpv positive patients nor to overall survival could be observed.	NA	3
Duncan et al,2013; PMID:23642549	USA	Oral Cavity	81	●	○	○	○	○	○	●	○	○	8.6	HPV16	8 to 27	positivity, 3+ staining(p16 IHC), and younger age bt not with survival	NA	2
Elanago et al,2011; PMID:21790221	India	Oral Cavity	60	●	○	○	○	●	○	●	●	○	50	HPV16	33	no statistically significant difference in the survival rate among cases with respect to	No correaltion	2
Stephen et al, 2013; PMID:23935769	USA	Oral Cavity & others*	20	●	○	○	○	○	○	●	○	○	50	HPV16	20	The prognostic effects of HPV16 and p16 alone were also analyzed for individual non-OP sites (oral cavity, larynx, hypopharynx), but were not	Both hpv16&p16 showed positive corelation when	1
Kouketsu et al, 2015, PMID:26711722	Japanease	Oral Cavity	174	●	○	○	○	●	○	●	○	○	7.4	HPV16	13.7	NA	No information	3
Walline et al, 2013; PMID:24177760	USA	Oral Cavity & others*	108	●	○	●	○	●	○	●	○	○	26	HPV16	18.9	NA	No information	3
Lingen et al, 2013; PMID:22841678	USA	Oral cavity	409	○	●	○	○	○	●	●	○	○	5.9	NA	NA	NA	No information	3

Reference	Cohort	Subsite	Patient No.(n)	Method/Marker of HPV detection									HPV DNA Prevelence	HPV subtype prevalence	p16 Prevalence	HPV DNA linked with ourcome	p16 linked with ourcome	
				HPV-DNA by PCR/qPCR/RFLP/sequencing	HPV Genotyping INNO-LiPA	PCR-Mass Array	DNA-PCR-Dot Blot	DNA by ISH	HPV-RNA by qRT-PCR (E2/E6/E7)	p16 IHC	E6/E7 IHC	E6/E7 antibody-ELISA						Comments
2013;Head Neck Oncol. 2013	Indian	OSMF & oral cavity	222	●	○	○	○	○	○	○	●	○	37.83	HPV16	NA	NA	No information	3
Rivero et al, 2006; PMID:16729170	Brazilian	Oral Cavity	40	●	○	○	○	○	○	○	○	○	0	NA	NA	NA	No information	3
Giuseppe et al,2012; PMID:22376902	Italy	Oral Cavity		●	○	○	○	●	○	●	○	○	11	NA	NA	NA	No information	3
Smith et al, 2004; PMID:14696105	USA	Oral Cavity & Others*	193	●	○	○	○	○	○	○	○	○	13.3	HPV 16	NA	HPV is not significant in oral cavity as compare to OPSCC.	No information	3
Herrero et al, 2003;PMID:14652239	France	Oral cavity & Others*	766	●	○	○	○	○	○	○	○	●	3.9	HPV16	NA	NA	NA	3
Kurose et al, 2004; PMID:15243477	Japanease	Oral cavity	662	●	○	○	○	○	○	○	○	○	0.6	HPV71 & HPV12	NA	NA	NA	3
Rice et al, 2000; PMID:10745235	London, UK	Oral Cavity	267	●	○	○	○	○	●	○	○	○	51.7	NA	NA	NA	NA	3
Lohaus et al,2014; PMID:25480095	Germany	Oral cavity & Others*	60	●	○	○	○	○	○	●	○	○	12	HPV 16	18.3	oropharyngeal carcinoma but not in the oral cavity	p16 showed a significant	2
al,2013;PMID:22907805	Canada	Oral cavity & Others*	49	○	○	○	○	○	○	●	○	○	NA	NA	13	NA	showed no association	2
al,2012;PMID:22747969	Taiwan	Oral cavity	103	○	●	○	○	○	○	○	○	○	30.1	HPV16	NA	aggressiveness, risk exposure, or the treatment outcome	NA	2
al,2012;PMID:22532307	Belgium	Oral cavity	162	●	○	○	○	●	○	●	○	○	44	HPV 16	53	withshorter disease-free survival in our series of 147 OSCCpatients.(neg coorealtion)	analyses did not show any impact	0
al,2012;PMID:22177275	Finland	Oral cavity & Others	37	○	●	○	○	○	○	○	○	○	41	HPV16	NA	survival. patients with LR-HPVs treated with radiotherapy had a poor prognosis.	NA	0
al,2012;PMID:21595750	Taiwan	Oral cavity	65		○	○	○	●	○	●	○	○	37	HPV 11	42	and bears a causally associated relationship different from other carcinogenic mechanisms.	NA	3
al,2015;PMID:26057833	Chile	Oral cavity	80	●	○	○	○	○	●	●	○	○	11	HPV 16&18	NA	No association with HPV presence	NA	2
al,2013;PMID:23743755	Colombia	Oral cavity & others	175	●	○	○	○	○	○	○	○	○	23.9	HPV 16&19	NA	NA	NA	3
al,2014;PMID:PMC4015165	India	Oral cavity	30	○	○	○	○	○	○	●	○	○	NA	NA	86.66	NA	between HPV and OSCC	3
Gichki et al,2012; PMID:22901210	Pakistan	Normal oral cavity	192	●	○	○	○	○	○	○	○	○	24.5	NA	NA	association between the presence of HPV and smoking	NA	3
al,2012;PMID:22104545	Taiwan	and verrucous lesions	31	●	○	○	○	●	○	○	○	○	28.3	hpv L1	NA	malignant transformation and disease-specific surviva	NA	3
Terai et al,1999;PMID:	Japan	normal cavity	37	●	●	○	○	○	○	○	○	○	81.1	HPV 5 subtypes	NA	NA	NA	3
Others*- represents studies that involve sites other than oral cavity																		
comments	0- represents studies that show HPV as a negative indicator of outcome																	
	1- represents studies that show HPV as a positive indicator of outcome																	
	2- represents studies that show HPV as a not an indicator of outcome																	
	3- studies where no information is available on HPV and outcome																	