

SUPPLEMENTARY MATERIALS

Genomic characterization of small cell carcinomas of the uterine cervix

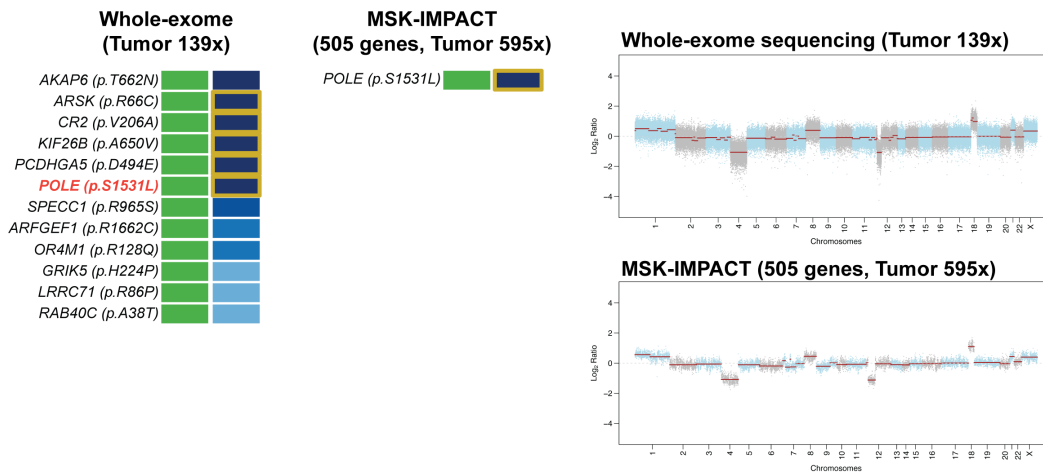
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Supplementary Figures S1 and S2

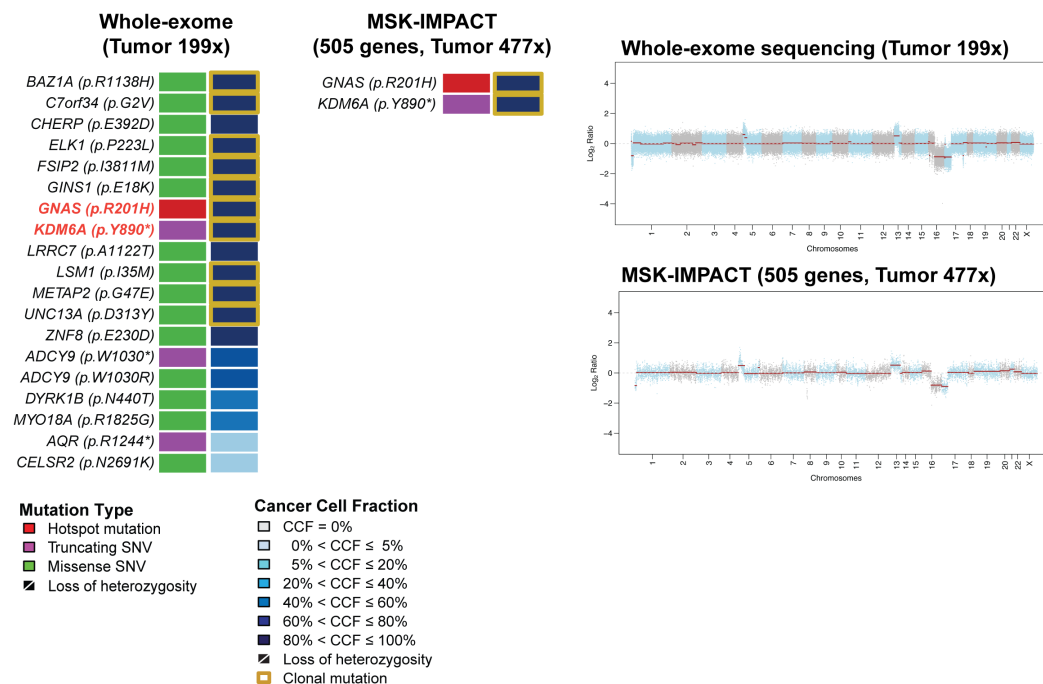
Supplementary Tables S1 - S6

Supplementary Figure S1

SCC1

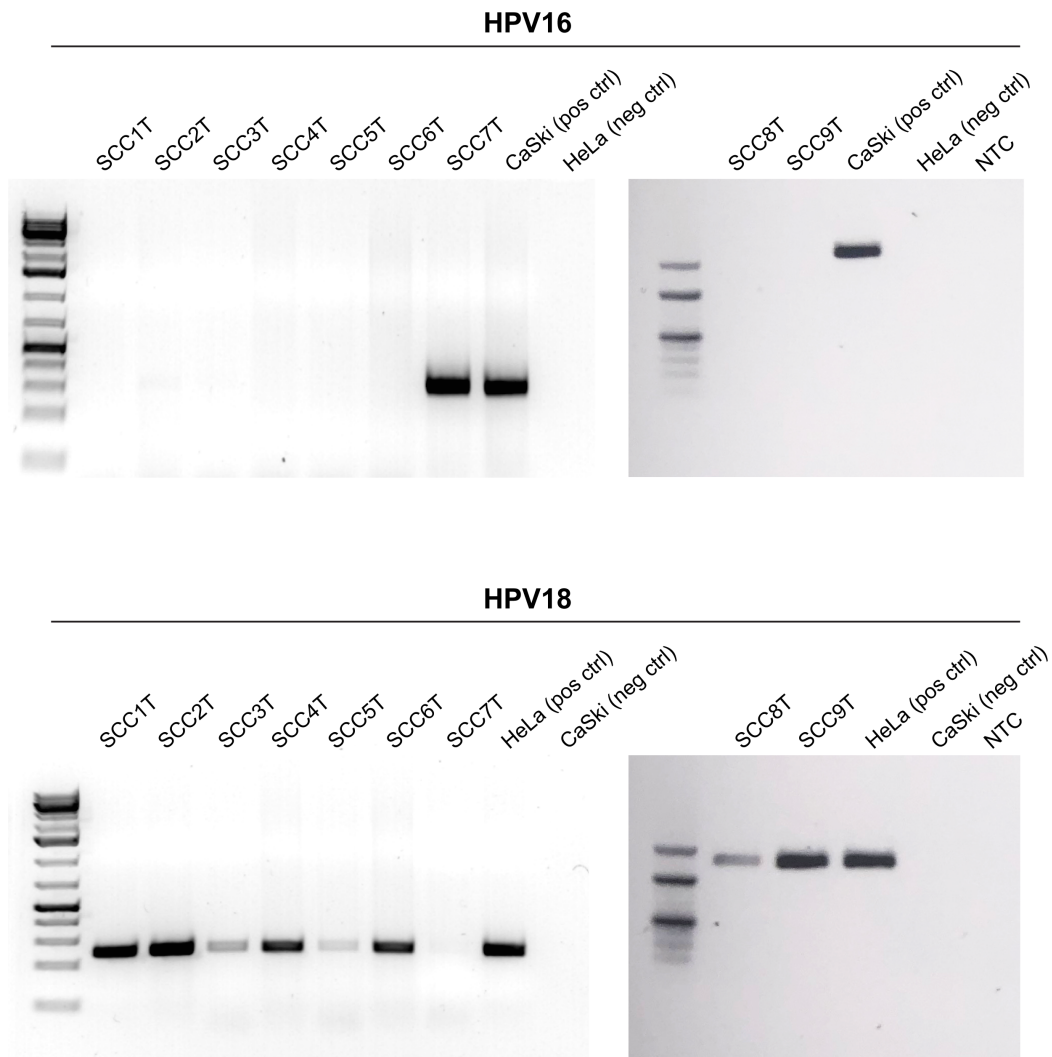


SCC4



Supplementary Figure S1: Comparison of whole-exome and high-depth targeted sequencing of uterine cervix small cell carcinomas SCC1 and SCC4. The same tumor and normal derived DNA samples of SCC1 (top) and SCC4 (bottom) were subjected to whole-exome sequencing and MSK-IMPACT sequencing targeting 505 cancer-related genes. The mutations identified by whole-exome sequencing are shown on the left, and genes being part of the 505 MSK-IMPACT panel are labeled in an orange font. All mutations identified by whole-exome sequencing affecting genes that were part of the 505 targeted panel were identified by MSK-IMPACT sequencing (middle), and no additional mutations were found. The copy number profiles of SCC1 and SCC4 based on whole-exome sequencing and MSK-IMPACT sequencing are also comparable.

Supplementary Figure S2



Supplementary Figure S2. Detection of HPV16 and HPV18 DNA in uterine cervix small cell carcinomas. DNA from uterine cervix small cell carcinomas was amplified with HPV16-specific primers (top) or with HPV18-specific primers (bottom). Agarose gel electrophoresis is shown. CaSki- and HeLa-derived cell line DNA, which are known to harbor HPV16 and HPV18, respectively, were included as positive controls. Ctrl, control. NTC, no template control.

Supplementary Table S1. Antibody clones, dilutions, antigen retrievals and scoring used for the immunohistochemical analyses performed.

Antibody	Clone	Dilution	Antigen retrieval	Detection	Company	Scoring
Ki67	MIB1	1:400	ER2, 30'	Leica Bond-RX-BOND Polymer Refine Detection	DAKO	Nuclear positivity in tumor cells; percentage counting 500 tumor cell nuclei.
p63	4A4	RTU	CC1, 24'	Ventana - OptiView	Ventana	No nuclear positivity allowed in the tumor cells.
Chromogranin A	LK2H10	RTU	CC1, 8'	Ventana - Optiview	Ventana	Positive cytoplasmic staining in tumor cells at any percentage.
Synaptophysin	Snp88	1:2000	CC1, 32'	Ventana - Optiview	Biogenex	Positive cytoplasmic staining in tumor cells at any percentage.
p16	E6H4	RTU	CC1, 32'	Ventana - Optiview	Ventana	Nuclear positivity and cytoplasmic positivity in tumor cells.
Pan-cytokeratin	Clone AE1/AE3	1:600	ER2, 30'	Ventana - Optiview	DAKO	Cytoplasmic positivity in tumor cells at any percentage considered as positive

RTU, ready to use.

Supplementary Table S2. Primers for Sanger sequencing validation of mutations identified by whole-exome sequencing.

Sample	Gene	AA change	Chromosome	Position	Forward	Reverse
SCC1T	AKAP6	T662N	14	3208695	GAATACCTAGCACTGCCCTCTC	CCTGCCTAGCCTTGTATGCT
SCC1T	ARSK	R66C	5	94901771	ATCAATGCTAATTAAATTTCTTTATTTCC	TGTAGCCACTTCTCCCATTC
SCC1T	KIF26B	A650V	1	245775129	CCCAGGCATGAGTGACAG	GAAGAACACGTGTGAGTTGCCG
SCC1T	POLE	S1531L	12	133219542	AATCGCTGGATGGCTCTG	CAGTGGGGAGTCTGCCCC
SCC1T	SPECC1	R965S	17	20163560	AGTATTAGCATCTTCTCCTTACCATGT	CAGGACTACTGCCCCAGGG
SCC2T	AGA	E130Q	4	178360736	CTTAGATCTGGTTTTATCCATGATTTT	TATGGATGTAGGAGCAGTAGGAGA
SCC2T	ARSF	C151*	X	3002330	TCACATGTTTTTGAATGTG	GAGCCAGAGCTGACTCTCAAA
SCC2T	BDP1	Q1353*	5	70806976	GGAAAACGAGCTAGAGGAGACC	TAAGTGTGTTGGACTTAAGGACAAA
SCC2T	COL5A1	R498S	9	137630654	TTGGACCTTGCCCTCGC	TGAAGACACAGCCCTCGGG
SCC2T	GLI3	R905H	7	42005957	GTCTTCAGGCTCATCTCTCC	GCTCGGCCTACCTGAGCA
SCC2T	OR2D3	R138W	11	6942644	GAGGAAAACCATTTCTTTTATGG	ACTAGTCCCCCACTGGCC
SCC2T	PIK3CA	E545K	3	178936091	AGGGGAAAAATGACAAAGAAAG	CTGCTTTATTTATCCAATAGGTATGG
SCC2T	SNRNP200	R1090W	2	96953698	TACATCGCTTTTTCGATCATC	GAGCTGGAGTAATGAAACCCG
SCC2T	TP53	R175H	17	7578406	AGACCTAAGAGCAATCAGTGAGGA	GCCAACTGGCCAAGACCT
SCC2T	UTP20	L583S	12	101696298	GTGGTTTCTCCTGTTTTTCTGC	GATTTCAACAATAGAGGCTTTT
SCC2T	ZNF584	E378K	19	58929017	CTGAAAGTCCACACTGGG	TCCTGAGCCTTCTGGA
SCC4T	CHERP	E392D	19	16639020	GTGGGATCTGGTGTGTG	TAAGTGCCTGCTACTGGATTAG
SCC4T	MYO18A	R1825G	15	72208836	GACTTGGTAATGCGATCTTGTAGT	GAAAACCTGGCTGAGAATTTTGG
SCC4T	LRRC7	A1122T	1	70504985	TTCTCAGCATCTGTGAATGAG	CGATCCAGCCCTCCCCTATA
SCC4T	GINS1	E18K	20	25388508	CGCCATGTTCTCGCAA	TGCCCTCCCAAGTCTAC
SCC4T	BAZ1A	R1138H	14	35234363	GACATATCTTGCAACGCGC	TTCTTCTGCTAGTCTATCCAAGTTT
SCC4T	METAP2	G47E	12	95868095	GAAGAAGGAGCTGCCTCTACG	TGAGCCAAACAGTTCAGCC
SCC4T	KDM6A	Y890*	X	44929570	AAACAGATCTGCTTCTGTTAAACC	TCAAACATTTTCACTTGTATGAACTT
SCC4T	ELK1	P223L	X	47497568	CTCTTCTTGGGCCCTTCTA	CCCGGGGAAATCACTG
SCC4T	ADCY9	W1030*	16	4016748	GGTCTTGGAGTAGGCTCGGG	ACTACCACGGAGACGTGGAA
SCC4T	ADCY9	W1030R	16	4016748	GGTCTTGGAGTAGGCTCGGG	ACTACCACGGAGACGTGGAA
SCC4T	GNAS	R201H	20	57484421	TATTACTGTTTCGGTGGCTTTG	ACAGTTGGCTTACTGGAAGTTGA
SCC4T	UNC13A	D313Y	19	17767038	TCTCTCTCCAGGAAGTC	CTCCGACATGGAGGATGAG
SCC5T	ANKRD44	P945S	2	197858397	CTGCAGTCCCTGTGACTTT	ATCCATGACTATTTGTAGAGTCAAGTAC
SCC5T	ROBO2	W451C	3	77607216	TTTGACAGATAGACCTCCACTATAA	AGATGATAACTCATGTACAAGTCCAAT
SCC5T	RQCD1	R259H	2	219457379	AGAATTTGTTTGTGTTTTGTGTGTTT	GTTCCAGCAGAGTATTTCCCTAAAGTC
SCC5T	FAM120A	T996S	9	96324527	TTTATTCATTAGGCGTCCAA	TGCCTGGTTTTGTAACTCT
SCC5T	FLNC	R1008H	7	128484151	GTGAACACACGAGGGGCT	TAGCGCACAGCCTGGG
SCC5T	PCDH17	L48F	13	58206822	TGCCCTCACTCTCAAGAACC	CTGTCTGGTCCACGTCC
SCC5T	ONECUT1	P249L	15	53081336	ATTGCTGACCTGCGCG	AACGGCTTCCGAAGCCC
SCC5T	CHAT	Q311P	10	50833698	GACACGCTGGTGCTCAG	GTAATGACGTGGAACGCATG
SCC5T	FZR1	M27V	19	3525875	CTGGGGGACACTCTCGG	GGGATGAAGCGGTCTCC
SCC5T	SCIN	V122M	7	12620694	GGCAGACACCTCATCAGTTTT	CGACCCITCACATGTAGGAG
SCC5T	ITSN1	P113S	21	35107500	CATAGCTATGAACTTATCAAAGTCAA	ATAATGTTATAAACCGAGAGGGAAAA
SCC5T	PLA2G4F	F318I	15	42442018	TGCTGCAGGGCCTTG	CACCCAGAACCCCCCA
SCC5T	CACNA1F	G985E	X	49074921	TGATCTGCGCCCGAGG	ATCTCGGTGGTGAAGATTCTG
SCC6T	IDH1	H309Y	2	209104653	GGGCTGCTTTGGAGAGCA	CTCTTCATGCAGTTGGACCC
SCC6T	LAMB4	R1252T	7	107696077	ATCAGTCCCTAAAGTCAACATAGG	TCTGTGAGGCAGACTTCAAAGA
SCC6T	METTL18	M1I	1	169762834	AGAACATTTTCTGCTCCTCTCTCT	CCATGTAAGCTGTAAAGGAGAATT
SCC6T	NIPBL	S2013I	5	37038770	GTTTCCACTAGCTTGTATATTTTAGTG	CTAAATTTAAAGCACTTAATGTTTTATGT
SCC6T	NOTCH2	E1025K	1	120483288	AGTCAATTACCTGACAGTTTTTCCC	TAGCTGATGTTGCTGTGTTTGC
SCC6T	NPY5R	F78L	4	164271657	GATGACTTACAGTATTTTCTGATTGGG	CATAATATGGCAGTACTTTGTC
SCC6T	ZCCHC11	D237N	1	52991244	GTAATTTAAATGGTTAAACCGGGG	TGCAAACTCACCCAGTACT
SCC6T	ZCCHC8	Q205*	12	122967884	AAGCCAAAAGTTTATGATTTTGGT	CTTTCCGAAGGATGGGAAA
SCC6T	RP11-1220K2.2	R356C	7	141838447	TCTTGGGTTCCAGCTTAGTCTG	TAAAATGGGAATTTGGTGTCAAC
SCC7T	C19orf57	E245*	19	14000936	CAGGGGACACCGTCTCC	CCTGTGACAGCAAGGGGC
SCC7T	CEP97	Q566E	3	101477146	AAAGAAACCATATCTCAAGCAACTTC	TCCTTATTTTATCAGTTAAGCAGACA
SCC7T	CEP97	S330*	3	101476002	TAGACCAGGTATTATGATTTTTAGGT	ATATGCACCTTTTTCTGCTTATGA
SCC7T	CEP97	Q580*	3	101477188	ATTTTAAACCCAGAGATCTGTTGCT	CAACCAACAAAACAGTGCATC
SCC7T	FGFR3	E140K	4	1801512	GGACCTGCCCCATCT	CCTTAGTCCCTCAGCTGCC
SCC7T	GBE1	H291Q	3	81692051	TGCTATCCCAAGATCATGAGTC	TCATTGTTAGCCGTTATGGAAC
SCC7T	IL24	E105K	1	207074845	GAAGATCCCTATCTCTGCTGTG	CCAGAGTAGAGATGACTTCCAGAGTC
SCC7T	KANK4	P795T	1	62728920	AGTGGGACACGCTGTAGTGA	GGAGGAACTCTTGCTGGC
SCC7T	NLRP14	C505*	11	7064772	ATGAAAACCTGCTATGTTTCAACC	CTTCATTTCAAAGGCCAAACA
SCC7T	P2RX7	E175Q	12	121600313	TTTGACCCCTATAGGAATTCAGAC	CTGAGAGGGGAAGGCC
SCC7T	RNF168	Q105*	3	196215543	ACTAAATTTAAATCTGAAACTAAAAACACA	CAAAAGGAGATATTGCTGCAGTT
SCC7T	SLC12A7	E989Q	5	1057647	AGGATCTGTTCCCCCGAG	CAGGAACACCCGCTCC
SCC7T	TMEM51	L213F	1	15546114	TCACTGACGGGGCTCG	CGTGGCTCAAGTGGGG
SCC7T	INPP5D	L46Q	2	234104059	CTGCAAATGGAACCCCTT	ACCCACGCCCACCTTAC
SCC7T	CACNA1A	R1977H	19	13325057	GTGGTGACATGCAAGCCA	AAGATCTACGAGCCATGATG

Supplementary Table S3. Sequencing statistics, number of somatic mutations and validation rates.

Case	Tissue Type	Sequencing Type	Mean Target Coverage (X)	Target Bases 2X	Target Bases 50X	Target Bases 100X	Total # Somatic Mutations	# Somatic Non-synonymous Mutations	Validation Rate Sanger Sequencing	Validation Rate MSK-IMPACT
SCC1	Normal	Whole-exome	88	100%	77%	34%	21	12	100% (5/5)	100% (1/1)
	Tumor		139	100%	92%	68%				
SCC2	Normal	Whole-exome	121	100%	98%	93%	34	24	100% (11/11)	Not performed
	Tumor		336	100%	90%	59%				
SCC3	Normal	MSK-IMPACT 505	122	95%	78%	56%	10	6	Not performed	Not performed
	Tumor		408	100%	99%	99%				
SCC4	Normal	Whole-exome	152	100%	96%	84%	32	19 (18 validated)	92% (11/12)	100% (2/2)
	Tumor		199	100%	95%	75%				
SCC5	Normal	Whole-exome	140	100%	97%	88%	41	26	100% (13/13)	Not performed
	Tumor		221	100%	93%	69%				
SCC6	Normal	Whole-exome	181	100%	93%	73%	42	26	100% (9/9)	Not performed
	Tumor		182	100%	96%	80%				
SCC7	Normal	Whole-exome	175	100%	91%	70%	84	59 (57 validated)	87% (13/15)	Not performed
	Tumor		183	100%	94%	75%				
SCC8	Normal Pool	MSK-IMPACT 505	115	100%	79%	55%	0	0	Not applicable	Not performed
	Tumor		278	99%	99%	97%				
SCC9	Normal	MSK-IMPACT 505	202	100%	99%	93%	10	8	Not performed	Not performed
	Tumor		405	100%	99%	99%				

Supplementary Table S5. Putative in-frame fusion transcripts with driver probability >0.1 identified in SCC1 and SCC2 using RNA-sequencing.

Sample ID	Genes	Genomic coordinates	Spanning and split reads (n)	Split reads (n)	Driver probability (Oncofuse)	Algorithm	5' location	3' location	P-value
SCC2T	VMP1-RPS6KB1	chr17:57842500>chr17:57987922	3	3	0.997438	chimerascan	Intron	Intron	0.04913752
SCC2T	SLC35G2-NCK1	chr3:136538057>chr3:136646825	2	1	0.9961532	chimerascan	Exon	Intron	0.05754801
SCC1T	DMC1-DDX17	chr22:38917612>chr22:38897286	3	2	0.9858879	chimerascan	Intron	Intron	0.14232601
SCC1T	ABCA10-ABCA6	chr17:67145173>chr17:67136890	2	2	0.9736921	chimerascan	Intron	Intron	0.19960184
SCC1T	ARFIP2-TRIM3	chr11:6498947>chr11:6486963	2	2	0.9446882	chimerascan	Intron	Intron	0.34136487
SCC2T	PDLIM2-C8orf58	chr8:22447255>chr8:22458394	2	2	0.8289519	chimerascan	Intron	Intron	0.63653723
SCC2T	GKAP1-KIF27	chr9:86431910>chr9:86530594	7	2	0.5981402	chimerascan	Intron	Intron	1
SCC2T	GKAP1-KIF27	chr9:86431910>chr9:86530594	28	17	0.5981402	defuse	Intron	Intron	1
SCC1T	POLA2-CDC42EP2	chr11:65063462>chr11:65088014	4	3	0.5912114	chimerascan	Intron	Intron	1
SCC2T	POLA2-CDC42EP2	chr11:65063462>chr11:65088014	23	10	0.5912114	chimerascan	Intron	Intron	1
SCC2T	POLA2-CDC42EP2	chr11:65063462>chr11:65088014	41	21	0.5912114	defuse	Intron	Intron	0.97330609
SCC2T	PIBF1-KLF5	chr13:73573134>chr13:73635998	2	2	0.5481406	chimerascan	Intron	Intron	1
SCC1T	TLK2-FAM157A	chr17:60637488>chr3:197907625	33	33	0.2722771	chimerascan	Intron	Intron	1
SCC2T	TLK2-FAM157A	chr17:60637488>chr3:197907625	44	29	0.2722771	chimerascan	Intron	Intron	1
SCC1T	TLK2-FAM157B	chr17:60637488>chr9:141134069	33	33	0.2722771	chimerascan	Intron	Intron	1
SCC2T	HOXB6-HOXB3	chr17:46681841>chr17:46651377	3	2	0.1747878	chimerascan	Intron	Exon	1
SCC1T	ZNF716-ZNF678	chr7:57522293>chr1:227842036	4	4	0.1540229	chimerascan	Intron	Intron	1
SCC1T	F8-TMLHE	chrX:154114408>chrX:154718952	2	1	0.1096824	chimerascan	Intron	Exon	1
SCC1T	MBD1-CCDC11	chr18:47799193>chr18:47788590	3	2	0.1056514	chimerascan	Intron	Intron	1

Supplementary Table S6. Putative HPV integration sites based on RNA-sequencing.

Sample ID	hg19 locus	hg19 annotation	Mate 1 HPV18 Position	Mate 2 hg19 Chromosome 18 Position
SCC1T	18q12.3	-	882	39411292
SCC1T	18q12.3	-	2280	39497187
SCC1T	18q12.3	-	3669	39401088
SCC2T	8p22	RP11-89M16.1-002 (lncRNA)	2603	129517175
SCC2T	8p22	RP11-89M16.1-002 (lncRNA)	4908	129518183
SCC2T	8p22	RP11-89M16.1-002 (lncRNA)	5996	129517009
SCC2T	8p22	RP11-89M16.1-002 (lncRNA)	5998	129509523