Supplementary Tables

Supplementary Table 1: Patient characteristics of 45 whole exome sequenced HNSCC tumors

Characteristics	Whole Exome Sequenced Tumors (n=45)	
Age, years		
Median (range)	61.0	(19-90)
Sex, N (%)		
Men	37	82.2%
Women	8	17.8%
Tumor Type, N (%)		
Primary	45	100.0%
Recurrent	0	0.0%
Clinical Disease Stage, N (%)		
I	1	2.2%
II	8	17.8%
III	7	15.6%
IV	29	64.4%
Tumor Site, N (%)		
Oral Cavity	24	53.3%
Oropharynx	3	6.7%
Hypopharynx	3 *	6.7%
Larynx	16	35.6%
Nasal Cavity	1*	2.2%
Tumor Grade, N (%)		
Well Differentiated	1	2.2%
Moderately Differentiated	32	71.1%
Poorly Differentiated	11	24.4%
Not evaluated	1	2.2%

* 2 patients with lesions at multiple sites.

Supplementary Table 1: Describes the clinical characteristics of the 45 new tumors analyzed in our cohort.

Supplementary Table 2A: Mutational events of PI3K pathway components in HNSCC tumors.

PI3K Pathway Component	Ref. GenBank Seq.	Tumor	Mutation Type	Genomic Change	Allele Change	Amino Acid Change
PIK3CA	NM_006218	HN_62415	Missense	g.chr3:178936092A>G	c.1791A>G	p.E545G
		HN_62426	Missense	g.chr3:178936091G>A	c.1790G>A	p.E545K
		HN_62469	Missense	g.chr3:178952085A>G	c.3297A>G	p.H1047R
		HN_62825	Missense	g.chr3:178916957G>T	c.501G>T	p.R115L
		HN_63027	Missense	g.chr3:178936082G>A	c.1781G>A	p.E542K
		HN_63039	Missense	g.chr3:178952085A>T	c.3297A>T	p.H1047L
		325	Missense	g.chr3:180434779A>T	c.3297T>A	p.H1047L
		HN11PT	Missense	g.chr3:180434779A>G	c.3140A>G	p.H1047R
		HN41PT	Missense	g.chr3:180434779A>T	c.3140A>T	p.H1047L
		HNPTS_1	Missense	g.chr3:178936091G>A	c.1633G>A	p.E545K
		HNPTS_14	Missense	g.chr3:178922319G>C	c.1088G>C	p.G363A
		HNPTS_20	Missense	g.chr3:178948139T>C	c.2911T>C	p.C971R
		HNPTS_25	Missense	g.chr3:178952085A>G	c.3140A>G	p.H1047R
		HNPTS_26	Missense	g.chr3:178948153A>T	c.2925A>T	p.R975S
		HNPTS_29	Missense	g.chr3:178936082G>A	c.1624G>A	p.E542K
		HNPTS_35	Missense	g.chr3:178936082G>A	c.1624G>A	p.E542K
		HNPTS_38	Missense	g.chr3:178952085A>G	c.3140A>G	p.H1047R
		HNPTS_42	Missense	g.chr3:178936091G>A	c.1633G>A	p.E545K
		HNPTS_45	Missense	g.chr3:178952085A>G	c.3140A>G	p.H1047R
PIK3CG	NM_002649	HN_01000	Missense	g.chr7:106545584C>A	c.3146C>A	p.R1021S
		HN_62532	Missense	g.chr7:106520100T>A	c.2613T>A	p.L843H
		HN_62854	Missense	g.chr7:106509343C>T	c.1422C>T	p.S446F
		HN_63021	Missense	g.chr7:106509582C>T	c.1661C>T	p.P526S
		HN22PT	Missense	g.chr7:106296714G>A	c.1472G>A	p.G491E
		HNPTS_42	Missense	g.chr7:106508473C>T	c.467C>T	p.A156V
PTEN	NM_000314	HN_00190	Missense	g.chr10:89692792C>G	c.1307C>G	p.D92E
		HN_62652	Splice_Site_SNP	g.chr10:89712017G>A	c.e7_splice_site	
		HN_62741	Missense	g.chr10:89717729G>T	c.1785G>T	p.D252Y
		HN_62863	Missense	g.chr10:89717712C>T	c.1768C>T	p.P246L
		HN_63039	Nonsense	g.chr10:89720852C>T	c.2034C>T	p.R335*
		HNPTS_1	Missense	g.chr10:89624268G>T	c.42G>T	p.R14S
PIK3R5	NM_014308	HNPTS_16	Missense	g.chr17:8792082G>A	c.1022C>T	p.A341V
		HNPTS_22	Missense	g.chr17:8808132T>C	c.374A>G	p.E125G
		HNPTS_29	Missense	g.chr17:8792140C>T	c.964G>A	p.E322K
		HNPTS_38	Nonsense	g.chr17:8812417C>A	c.178G>T	p.E60*
PIK3AP1	NM_152309	HN_62506	Missense	g.chr10:98469347G>T	c.535C>A	p.A136D
		91	Missense	g.chr10:98378183C>T	c.1433G>A	p.R478Q
		266	Missense	g.chr10:98398536G>C	c.1055C>G	p.T352S
		HN22PT	Missense	g.chr10:98401046C>G	c.937G>C	p.G313R
PIK3R1	NM_181523	HN_00361	In_frame_Ins	g.chr5:67589591_67589592insATA	c.1914_1915insATA	p.453_454insN
		HN_62338	Missense	g.chr5:67576786A>G	c.1428A>G	p.I290V
		HN_62421	Missense	g.chr5:67591085G>C	c.2238G>C	p.D560H
		HNPTS_6	Nonsense	g.chr5:67591278_67591279GA>CT	c.1776_1777GA>CT	p.592_593KK>N*
PIK3C2G	NM_004570	HN_00190	Missense	g.chr12:18534785G>T	c.2044G>T	p.V656L
		HNPTS_23	Frame_Shift_Ins	g.chr12:18435201_18435202insT	c.186_187insT	p.T62fs
		HNPTS_29	Missense	g.chr12:18719918C>T	c.3815C>T	p.S1272L
MTOR	NM_004958	HN_62421	Missense	g.chr1:11182067A>T	c.6900T>A	p.L2260H
		HN_62469	Missense	g.chr1:11272448C>T	c.3603G>A	p.R1161Q
PIK3C2A	NM_002645	HN_62699	Splice_Site_SNP	g.chr11:17167490C>T	c.e6_splice_site	
PIK3C2B	NM_002646	HN_62739	Missense	g.chr1:204426879G>A	c.2169C>T	p.R564C
PIK3CD	NM_005026	HN_62672	Missense	g.chr1:9780202A>T	c.1475A>T	p.T423S
PIK3R6	NM_001010855	HN_62860	Missense	g.chr17:8730556C>T	c.1688G>A	p.R483H
PIK3IP1	NM_052880	HNPTS_1	Missense	g.chr22:31685563C>A	c.430G>T	p.A144S
AKT2	NM_001626	HNPTS_45	Missense	g.chr19:40741920T>C	c.1052A>G	p.Y351C
TSC1	NM_000368	HN_00761	Nonsense	g.chr9:135796754G>A	c.967C>T	p.R245*
TSC2	NM_000548	HNPTS_42	Nonsense	g.chr16:2134999C>G	c.4541C>G	p.S1514*
RICTOR	NM_152756	HNPTS_18	Nonsense	g.chr5:38944564C>A	c.4897G>T	p.E1633*
		HNPTS_27	Missense	g.chr5:38991111C>G	c.523G>C	p.D175H
RPTOR	NM_020761	HNPTS_17	Missense	g.chr17:78820280C>T	c.1220C>T	p.P407L

Supplementary Table 2B: Mutational events of MAPK pathway components in HNSCC tumors.

MAPK Pathway Component	Ref. GenBank Seq.	Tumor	Mutation Type	Genomic Change	Allele Change	Amino Acid Change
HRAS	NM_005343	HN_00466	Missense	g.chr11:533875G>T	c.369C>A	p.Q61K
	NM_005343	HN_62469	Missense	g.chr11:534285C>A	c.226G>T	p.G13V
	NM_005343	HN_62863	Missense	g.chr11:534288C>G	c.223G>C	p.G12A
	NM_005343	HN_63080	Missense	g.chr11:534288C>T	c.223G>A	p.G12D
	NM_001130442	HN11PT	Missense	g.chr11:523874T>A	c.182A>T	p.Q61L
		HN12PT	Missense	g.chr11:524286C>G	c.37G>C	p.G13R
		166	Missense	g.chr11:524288C>T	c.35G>A	p.G12D
KRAS	NM_033360	HN_62421	Missense	g.chr12:25378557C>G	c.622G>C	p.K147N
	NM_033360	HNPTS_23	Missense	g.chr12:25368473T>C	c.472A>G	p.T158A
RAF1	NM_002880	478	Missense	g.chr3:12601123G>C	c.1837C>G	p.L613V
SHC2	NM_012435	HNPTS_26	Missense	g.chr19:436383T>C	c.823A>G	p.R275G
SHC3	NM_016848	HNPTS_4	Missense	g.chr9:91656972C>A	c.1329G>T	p.E443D

Supplementary Table 2C: Mutational events of the JAK/STAT pathway components in HNSCC tumors.

JAK/STAT Pathway Component	Ref. GenBank Seq.	Tumor	Mutation Type	Genomic Change	Allele Change	Amino Acid Change
JAK1	NM_002227	HNPTS_14	Missense	g.chr1:65335021T>A	c.620A>T	p.Q207L
		HNPTS_18	Missense	g.chr1:65307005C>G	c.2572G>C	p.E858Q
JAK2	NM_004972	HNPTS_17	Missense	g.chr9:5054676G>A	c.728G>A	p.C243Y
JAK3	NM_000215	HNPTS_20	Missense	g.chr19:17949121T>A	c.1520A>T	p.Q507L
		HN_62376	Nonsense	g.chr19:17955190G>A	c.137C>T	p.Q13*
		HN_63080	Missense	g.chr19:17942173G>A	c.2942C>T	p.R948C
		HN33PT	Missense	g.chr19:17812143A>T	c.1150T>A	p.F384I
STAT1	NM_007315	HN_63080	Missense	g.chr2:191856003G>T	c.1376C>A	p.Q330K
	NM_139266	388	Missense	g.chr2:191548805C>T	c.2113G>A	p.E705K
STAT3	NM_139276	HN_01000	Missense	g.chr17:40498671C>G	c.429G>C	p.E63D
STAT5B	NM_012448	HN_62338	Missense	g.chr17:40375520T>A	c.599A>T	p.I144F
IL6ST	NM_002184	HN_62415	Missense	g.chr5:55247378T>A	c.2009A>T	p.D585V
		HN_62469	Missense	g.chr5:55250817G>A	c.1526C>T	p.T424I
		HNPTS_21	Missense	g.chr5:55250717C>A	c.1371G>T	p.W457C
IL6R	NM_000565	HNPTS_1	Missense	g.chr1:154427027G>C	c.1130G>C	p.G377A

Supplementary Tables 2A-2C: Describe the mutations observed in the defined mitogenic pathways, as observed in our cohort.

Supplementary Table 2D: Average mutation rate of the PI3K, MAPK and JAK/STAT pathways in the HNSCC cohort (N=151).

Mutated Pathways	Gene Length covered (bases)	Total No. of Mutations	Average Mutation Rate per Targeted Base ($x10^6$)	P-value (vs. Background HNSCC mutation rate[#])
PI3K Pathway	79994	59	5.9	***0.0055***
MAPK Pathway	19135	12	7.2	***0.0133***
JAK/STAT Pathway	35228	15	2.4	0.4931

Supplementary Table 2E: Average mutation rate of mutated genes in the PI3K, MAPK and JAK/STAT pathways in the HNSCC cohort (N=151).

Mutated Components	Genes	Gene Length covered (bases)	Total No. of Mutations	Average Mutation Rate per Targeted Base ($x10^6$)	P-value (vs. Background HNSCC mutation rate[#])
PI3K Pathway	***PIK3CA***	3287	19	38.3	***< 0.0001***
	PIK3CG	3349	6	11.9	0.0647
	PTEN	1248	6	31.8	***0.0253***
	PIK3R5	2715	4	9.7	0.1629
	PIK3AP1	2486	4	10.7	0.1477
	PIK3R1	2365	4	11.2	0.1398
	PIK3C2G	4462	3	4.5	0.5621
	MTOR	7878	2	1.7	0.3745
	PIK3C2A	5189	1	1.3	0.2651
	PIK3C2B	5033	1	1.3	0.2881
	PIK3CD	3223	1	2.1	0.6932
	PIK3R6	2340	1	2.8	0.9772
	PIK3IP1	816	1	8.1	0.524
	AKT2	1498	1	4.4	0.7361
	TSC1	3579	1	1.8	0.5916
	TSC2	5588	1	1.2	0.2127
	RICTOR	5279	2	2.5	0.8302
	RPTOR	4144	1	1.6	0.4522
MAPK Pathway	***HRAS***	653	7	71.0	***0.0106***
	KRAS	707	2	18.7	0.2333
	RAF1	2011	1	3.3	0.9104
	SHC2	1797	1	3.7	0.8374
	SHC3	1833	1	3.6	0.8486
JAK/STAT Pathway	***JAK1***	3561	2	3.7	0.7658
	JAK2	3491	1	1.9	0.6155
	JAK3	3467	4	7.6	0.2213
	STAT1	2349	2	5.6	0.5
	STAT3	2405	1	2.8	0.9562
	STAT5B	2436	1	2.7	0.9464
	IL6ST	2817	3	7.1	0.3147
	IL6R	1447	1	4.6	0.7197

[#] *P value was calculated by comparing the average mutation rate of each pathway gene in this cohort to the background mutation rate per targeted base for all genes in the COSMIC database for upper-aerodigestive tract cancers.*

Supplementary Tables 2D-2E: Demonstrate the statistical significance of the mutation rates observed in the defined mitogenic pathways, and individual genes compared to HNSCC background mutation rates, normalized to bases covered by exome sequencing in our cohort.

Supplementary Table 3: Association of cancer gene mutation with PI3K-mutated HNSCC tumors.

Gene	PI3K-Mutated Tumors		PI3K WT Tumors		P value
	No. of Tumors with Mutation	Freq (%)	No. of Tumors with Mutation	Freq (%)	
MLL	4/46	8.7%	0/105	0.0%	0.007841
ARID1A	3/46	6.5%	0/105	0.0%	0.026988
MLL3	7/46	15.2%	5/105	4.8%	0.045901
ATRX	4/46	8.7%	2/105	1.9%	0.07014
TRIM24	4/46	8.7%	2/105	1.9%	0.07014
CDKN2A	11/46	23.9%	12/105	11.4%	0.082421
AKAP9	3/46	6.5%	1/105	1.0%	0.084428
EP300	3/46	6.5%	1/105	1.0%	0.084428
ERCC5	3/46	6.5%	1/105	1.0%	0.084428
BRCA1	2/46	4.3%	0/105	0.0%	0.091391
EPS15	2/46	4.3%	0/105	0.0%	0.091391
FAM123B	2/46	4.3%	0/105	0.0%	0.091391
GAS7	2/46	4.3%	0/105	0.0%	0.091391
HIST1H4H	2/46	4.3%	0/105	0.0%	0.091391
JAK1	2/46	4.3%	0/105	0.0%	0.091391
KDM5A	2/46	4.3%	0/105	0.0%	0.091391
KRAS	2/46	4.3%	0/105	0.0%	0.091391
LPP	2/46	4.3%	0/105	0.0%	0.091391
MLLT4	2/46	4.3%	0/105	0.0%	0.091391
PAX3	2/46	4.3%	0/105	0.0%	0.091391
RET	2/46	4.3%	0/105	0.0%	0.091391
SF3B1	2/46	4.3%	0/105	0.0%	0.091391
SRGAP3	2/46	4.3%	0/105	0.0%	0.091391
TRIP11	2/46	4.3%	0/105	0.0%	0.091391
ZMYM2	2/46	4.3%	0/105	0.0%	0.091391

Supplementary Table 3: Demonstrates the correlation between PI3K mutant status and mutations in other cancer genes as defined by COSMIC, and the respective statistical significance of each correlation.

Supplementary Table 4: PI3K pathway mutations of HPV-positive HNSCC tumors.

HPV-Positive HNSCC Tumors	PI3K Pathway Mutation
HN_00361 *	*PIK3R1* (453_454insN)
HN_00466	NO
HN_62318	NO
HN_62740	NO
HN_62825	*PIK3CA* (R115L)
HN_63027 *	*PIK3CA* (E542K)
HN_63115	NO
HN_62810	NO
HN_00338	NO
HN_62374	NO
HN_62426	*PIK3CA* (E545K)
HN19PT	NO
HN20PT	NO
HN41PT *	*PIK3CA* (H1047L)
HN42PT	NO

** denotes tumors with PI3K pathway mutations as the only cancer gene mutations.*

Supplementary Table 4: Describes the PI3K mutational status of HPV+ tumors in our cohort.

Supplementary Table 5: Distribution of *PIK3CA* mutations in multiple cancers.

Cancer Types	*PIK3CA* Domain Mutations				
	ABD	RBD	C2	Helical	Kinase
HNSCC (n=151)*	1/19 (5.26%)	0/19 (0%)	1/19 (5.26%)	7/19 (36.84%)	10/19 (52.63%)
LUNG SCC (n=179)**	5/31 (16.13%)	0/31 (0.00%)	4/31 (12.90%)	16/31 (51.61%)	6/31 (19.35%)
CERVICAL SCC (n=35)	0/9 (0.00%)	0/9 (0.00%)	1/9 (11.11%)	5/9 (55.56%)	3/9 (33.33%)
OVARIAN SEROUS CYSTADENOCARCINOMA (n=440)	1/3 (33.33%)	0/3 (0.00%)	0/3 (0.00%)	1/3 (33.33%)	1/3 (33.33%)
ENDOMETRIOID CARCINOMA (n=240)***	49/164 (29.88%)	2/164 (1.22%)	26/164 (15.85%)	43/164 (26.22%)	43/164 (26.22%)
BREAST INVASIVE CARCINOMA (n=482)†	11/190 (5.79%)	0/190 (0.00%)	20/190 (10.53%)	67/190 (35.26%)	92/190 (48.42%)
PROSTATE ADENOCARCINOMA (n=82)	0/2 (0.00%)	0/2 (0.00%)	1/2 (50.00%)	1/2 (50.00%)	0/2 (0.00%)
KIDNEY RENAL CLEAR CELL CARCINOMA (n=43)††	2/10 (20.00%)	0/10 (0.00%)	0/10 (0.00%)	4/10 (40.00%)	3/10 (30.00%)
STOMACH ADENOCARCINOMA (n=132)‡	5/33 (15.15%)	1/33 (3.03%)	6/33 (18.18%)	14/33 (42.42%)	7/33 (21.21%)
GLIOBLASTOMA MULTIFORME (n=283)	11/27 (40.74%)	0/27 (0.00%)	4/27 (14.81%)	7/27 (25.93%)	5/27 (18.52%)
LUNG ADENOCARCINOMA (n=172)▲	3/11 (27.27%)	0/11 (0.00%)	2/11 (18.18%)	5/11 (45.45%)	1/11 (9.09%)
COLON AND RECTAL ADENOCARCINOMA (n=212)	6/51 (11.76%)	1/51 (1.96%)	12/51 (23.53%)	19/51 (37.25%)	13/51 (25.49%)
BLADDER UROTHELIAL CARCINOMA (n=25)	0/3 (0.00%)	0/3 (0.00%)	0/3 (0.00%)	1/3 (33.33%)	2/3 (66.67%)

Footnotes: Domains are defined as in Figure 3A. Mutations closest to nearby domains *AA 115 , 169, 498; **AA 118, 320, 495; ***AA 110(n=3), 111(n=4), 113, 115, 118(n=5), 170, 495, 522; 1 splice site unclassified; †c.383, AA 109, 110, 111(n=2), 118(n=2), 512 ; †† 1 mutation unclassified; ‡AA 111, 179, 512; ▲AA 111. Total *PIK3CA* mutations in each cancer type are represented in the denominator for each cancer type. Data extracted from cBio portal.

Supplementary Table 5: Describes the distribution of PIK3CA mutations across cancer types.