

Table SI. Enrichment of quantitative PCR primer pairs.

DNA type	Primer name	Gene specificity	Amplified locus	Amplicon length, bp
Methylated DNA	Methylated DNA control (primer pair no. 1)	Non-specific	Chr1:30089133-30089053	81
Unmethylated DNA	Unmethylated DNA control (primer pair no. 2)	Non-specific	chr3: 20085460-20085556	92
Gene-specific methylated DNA	Methylated human DNA region	<i>TSH2B</i>	chr6:25835060-25835229	170
Gene-specific unmethylated DNA	Unmethylated human DNA region	<i>GAPDH</i>	chr12:6,513,903-6,513,966	64

Table SII. Candidate gene primers and probe sequences for quantitative methylation-specific PCR assay.

Official Symbol	Gene ID	Forward (5'-3')	Probe 6FAM (5'-3')TAMRA	Reverse (5'-3')	(Ref.)
<i>ACTB</i>	60	TGGTGATGGAGGAGGTTTAA TAAGT	ACCACCACCCAACACACAATAACA AACACA	AACCAATAAAACCTACTCCTCCCTTAA	(36)
<i>CALCA</i>	796	GTTTTGGAAGTATGAGGGTG ACG	ATTCCGCCAATACACAACAACCAAT AAACG	TTCCCGCCGCTATAAATCG	(36)
<i>CDHI</i>	999	AATTTTAGGTTAGAGGGTTAT CGCGT	CGCCACCCGACCTCGCAT	TCCCCAAAACGAAACTAACGAC	(36)
<i>DAPK1</i>	1612	GGATAGTCGGATCGAGTTAA CGTC	TTCGGTAATTCGTAGCGGTAGGGTT TGG	CCCTCCCAAACGCCGA	(36)
<i>SFRP1</i>	6422	GAATTCGTTTCGCGAGGGA	CCGTCACCGACGCGAAAACCAAT	AAACGAACCGCACTCGTTACC	(36)
<i>MAGI2</i>	9863	GCGCGTTATAAGTTTAGGTTT C	AACTCCCTCCGACCTAAACTCCG	CTTCTTCAACCTCTATACGAC	(37-39)
<i>PAX1</i>	5075	TCGTTAGGGAGAAAGGAATT TGT	TTTCGTGCGTTCGCGTTTGGG	TAAATCCGACGCCCTCCTA	(40)
<i>PAX5</i>	5079	GCGTAAGAGAGACGAAGGTA AG	AGAGGTTTCGCGTAGTTTCGTCGG	ATATTCGCGAACACCTCTACTAC	(40)
<i>PITX2</i>	5308	GGAGTGACGTGACGTTAGTA GAGATTT	CGCCCGCGCGCCACTATACA	AACCGCGCAACCGAACT	(41)
<i>SOCS1</i>	8651	GCGTCGAGTTCGTGGGTATTT	ACAATTCGCTAACGACTATCGCGC A	CCGAAACCATCTTCACGCTAA	(42)
<i>TIMP3</i>	7078	GCGTCGGAGGTTAAGGTTGT T	AACTCGCTCGCCCGCCGAA	CTCTCCAAAATTACCGTACGCG	(43)

Table VIII. Common differentially methylated regions across all three head and neck squamous cell carcinoma sub-sites after The Cancer Genome Atlas and UCSC Cancer Genomics Browser confirmation.

Gene ID	Peak value	Biological pathway interaction
<i>CREB3</i>	5.610	cGMP-PKG signaling pathway, cAMP signaling pathway, PI3K-Akt signaling pathway, AMPK signaling pathway, TNF signaling pathway, thyroid hormone synthesis, alcoholism, viral carcinogenesis, prostate cancer
<i>PLCE1</i>	5.593	Inositol phosphate metabolism, metabolic pathways, Ras signaling pathway, Rap1 signaling pathway, calcium signaling pathway, cAMP signaling pathway, phosphatidylinositol signaling system, thyroid hormone signaling pathway, proteoglycans in cancer
<i>RAB5C</i>	4.928	Ras signaling pathway, endocytosis, phagosome
<i>PRB2</i>	4.875	Salivary secretion
<i>NUTF2</i>	4.778	Protein import into the nucleus
<i>ZNF628</i>	4.589	Zinc finger protein regulating transcription
<i>GPR31</i>	4.567	G-protein coupled receptor
<i>PIK3R6</i>	4.493	Produces the lipid second messenger phosphatidylinositol 3,4,5-trisphosphate
<i>EIF6</i>	4.490	Ribosome biogenesis in eukaryotes
<i>MTA1</i>	4.336	Downregulated in estrogen receptor <sup>+</sup> breast tumors
<i>CHEK2</i>	3.937	Cell cycle, p53 signaling pathway
<i>NRTN</i>	3.823	Ligand of the TGF- $\beta$ superfamily of proteins
<i>PITPNM2</i>	3.739	Membrane-associated phosphatidylinositol transfer domain-containing proteins
<i>EIF3J</i>	3.484	RNA transport
<i>IL34</i>	3.459	Promotes differentiation and viability of monocytes and macrophages
<i>DUSP5</i>	3.243	MAPK signaling pathway
<i>TOP1</i>	3.057	DNA topoisomerase
<i>PRKD1</i>	2.991	Rap1 signaling pathway
<i>PGS1</i>	2.828	Glycerophospholipid metabolism, metabolic pathways
<i>RASSF1</i>	2.8084	Ras signaling pathway, Hippo signaling pathway, pathways in cancer, microRNAs in cancer, bladder cancer, non-small cell lung cancer
<i>CCL13</i>	2.766	Cytokine-cytokine receptor interaction, chemokine signaling pathway, NF- $\kappa$ B signaling pathway
<i>ZNF395</i>	2.681	RNA polymerase II core promoter proximal region sequence-specific DNA binding
<i>MLH1</i>	2.643	Mismatch repair, pathways in cancer, colorectal and endometrial cancer
<i>HOXA9</i>	2.583	Homeobox gene
<i>CDKN2A</i>	2.3585	Cell cycle, p53 signaling pathway, pathways in cancer, viral carcinogenesis, microRNAs in cancer, pancreatic cancer, glioma, melanoma, bladder cancer, chronic myeloid leukemia, non-small cell lung cancer
<i>DAPK1</i>	2.416	Pathways in cancer, bladder cancer
<i>FOXI1</i>	2.342	Forkhead family of transcription factors, role in development and embryogenesis
<i>NOTCH3</i>	2.341	Dorso-ventral axis formation, notch signaling pathway, thyroid hormone signaling pathway, microRNAs in cancer
<i>HSBP1</i>	2.287	Heat shock factors
<i>HOXA6</i>	2.165	Homeobox gene
<i>CHAT</i>	2.114	Glycerophospholipid metabolism, cholinergic synapse
<i>BRAP</i>	2.056	Ras signaling pathway

Table SIV. Prevalence of aberrant methylation of candidate genes in HNSCC (n=50) and normal samples (n=7).

	<i>DAPK1</i>			<i>CDH1</i>			<i>PAX1</i>			<i>CALCA</i>			<i>TIMP3</i>		
	HNSCC, n	Normal, n	P-value	HNSCC, n	Normal, n	P-value	HNSCC, n	Normal, n	P-value	HNSCC, n	Normal, n	P-value	HNSCC, n	Normal, n	P-value
Methylated	29	0		25	1		41	0		22	0		38	1	
Unmethylated	21	7	0.005	25	6	0.112	9	7	<0.001	28	7	0.036	12	6	0.003

HNSCC, head and neck squamous cell carcinoma.