Supplementary Table 1. NSD1 mutations in samples included in the H3K36 cluster. Hugo Symbol NSD1, Chromosome 5.

Sample ID	Protein Change	Mutation Type	Start Position	End Position	Reference Allele	Variant Allele	Allele Frequency	Mutation Assessor	Note
TCGA-BA-5555-01A-01D-1512-08	p.R788X	Nonsense	176637762	176637762	C	T	0.464286		
TCGA-CV-5441-01A-01D-1512-08	p.H1872fs	Frame_Shift_Del	176700778	176700779	AT	-	0.463415		
TCGA-CV-7409-01A-31D-2229-08	p.Q1153X	Nonsense	176638857	176638857	C	T	NA		
TCGA-BB-7870-01A-11D-2229-08	p.E990X	Nonsense	176638368	176638368	G	T	0.778325		
TCGA-BA-6869-01A-11D-1870-08	p.S707X	Nonsense	176637520	176637520	C	G	0.621212		
TCGA-CV-6960-01A-41D-2012-08	p.G1678W	Missense	176687055	176687055	G	T	0.309524	Medium	
TCGA-CV-7245-01A-11D-2012-08	p.E1534X	Nonsense	176675284	176675284	G	T	0.75		
TCGA-CR-7402-01A-11D-2012-08	p.G1524fs	Frame_Shift_Del	176675254	176675254	G	-	0.310345		
TCGA-CR-7402-01A-11D-2012-08	p.P2567T	Missense	176722068	176722068	C	A	0.1	Neutral	Sample also has NSD1 frame shift deletion and nonsense mutation
TCGA-CR-7402-01A-11D-2012-08	p.Q1989X	Nonsense	176709538	176709538	C	T	0.20339		
TCGA-CR-7402-01A-11D-2012-08	p.T723S	Missense	176637567	176637567	A	T	0.297619	Neutral	Sample also has NSD1 frame shift deletion and nonsense mutation
TCGA-CR-7364-01A-11D-2012-08	p.E1979X	Nonsense	176709508	176709508	G	T	0.336735		
TCGA-CV-7253-01A-11D-2012-08	p.S1086fs	Frame_Shift_Del	176638656	176638662	AGTAAAG	-	0.25		
TCGA-CR-7398-01A-11D-2012-08	p.S1528fs	Frameshift Insertion	176675268	176675268	-	A	0.0333333		
TCGA-CV-7250-01A-11D-2012-08	p.S1359X	Nonsense	176665392	176665392	C	A	0.692982		
TCGA-CV-7242-01A-11D-2012-08	p.A2009T	Missense	176710803	176710803	G	A	0.268041	High	
TCGA-CR-5247-01A-01D-2012-08	p.Y1997C	Missense	176709563	176709563	A	G	0.695652	High	
TCGA-CN-4727-01A-01D-1434-08	p.G1928X	Nonsense	176707725	176707725	G	T	0.222222		
TCGA-CN-4727-01A-01D-1434-08	p.G959X	Nonsense	176638275	176638275	G	T	0.235294		
TCGA-CN-4730-01A-01D-1434-08	p.S589fs	Frame_Shift_Del	176637167	176637168	TT	-	0.480519		
TCGA-CN-5356-01A-01D-1434-08	p.K1433X	Nonsense	176666861	176666861	A	T	0.439024		
TCGA-F7-7848-01A-11D-2129-08	p.M1094fs	Frame_Shift_Del	176638682	176638682	G	-	0.453125		
TCGA-CV-7440-01A-11D-2129-08	p.C1619S	Missense	176684042	176684042	G	C	0.448276	Medium	Sample also has NSD1 nonsense mutation
TCGA-CV-7440-01A-11D-2129-08	p.C792X	Nonsense	176637776	176637776	C	A	0.22449		
TCGA-CV-6941-01A-11D-1912-08	p.R1473X	Nonsense	176673717	176673717	C	T	0.578947		
TCGA-CV-A461-01A-41D-A25Y-08	p.R1757fs	Frame_Shift_Del	176694686	176694686	G	-	0.217687		
TCGA-CV-A461-01A-41D-A25Y-08	p.S981fs	frameshift insertion	176638343	176638343	-	G	0.159091		
TCGA-BA-A4IF-01A-11D-A25Y-08	p.P1665L	Missense	176687017	176687017	C	T	0.496	Medium	
TCGA-CN-A497-01A-11D-A24D-08	p.E1391X	Nonsense	176665487	176665487	G	T	0.325581		
TCGA-CN-A497-01A-11D-A24D-08	p.W2032X	Nonsense	176710873	176710873	G	A	0.382353		
TCGA-CN-A63W-01A-11D-A30E-08	p.W1160X	Nonsense	176638879	176638879	G	A	0.785714		
TCGA-CN-A641-01A-11D-A30E-08	p.C1897F	Missense	176707633	176707633	G	T	0.342105	High	Sample also has NSD1 frame shift insertion
TCGA-CN-A641-01A-11D-A30E-08	p.I1946fs	frameshift insertion	176707779	176707779	-	T	0.136364		
TCGA-CN-A641-01A-11D-A30E-08	p.R1948I	Missense	176707785	176707785	CG	AT	0.162162	Medium	Sample also has NSD1 frame shift insertion
TCGA-CN-A63T-01A-11D-A28R-08		Splice	176719160	176719160	G	A	0.2		
TCGA-CN-A63T-01A-11D-A28R-08	p.E1970A	Missense	176709482	176709482	A	C	0.190789	High	Sample also has NSD1 splicing mutation
TCGA-BB-A5HZ-01A-21D-A28R-08	p.G1095fs	Frame_Shift_Del	176638683	176638693	GCCACTTAA	-	0.215909		
TCGA-BB-A5HZ-01A-21D-A28R-08	p.I1291fs	Frameshift Insertion	176662896	176662896	-	T	0.2		
TCGA-P3-A5QF-01A-11D-A28R-08	p.R2005Q	Missense	176710792	176710792	G	A	0.59375	Medium	
TCGA-KU-A66S-01A-21D-A30E-08	p.H1616Y	Missense	176684032	176684032	C	T	0.348485	Medium	Sample also has NSD1 frame shift deletion
TCGA-KU-A66S-01A-21D-A30E-08	p.R1952fs	Frame_Shift_Del	176707798	176707798	G	-	0.363636		
TCGA-CN-A63U-01A-11D-A30E-08	p.E1501X	Nonsense	176675185	176675185	G	T	0.625		
TCGA-CQ-7072-01A-21D-A30E-08	p.Q679X	Nonsense	176637435	176637435	C	T	0.162791		
TCGA-BA-A6DL-01A-21D-A30E-08	p.R1072X	Nonsense	176638614	176638614	C	T	0.171598		
TCGA-BA-A6DL-01A-21D-A30E-08	p.R1200fs	Frame_Shift_Del	176638999	176639006	GGGATGAG	-	0.211268		
TCGA-H7-A6C5-01A-11D-A30E-08	p.1664_1665	nonFrame_Shift_Del	176687015	176687017	TCC	-	0.586207		
TCGA-D6-A6EO-01A-11D-A31L-08	p.E2028fs	Frame_Shift_Del	176710861	176710861	A	-	0.581633		
TCGA-D6-A74Q-01A-11D-A34J-08	p.P753fs	Frame_Shift_Del	176637659	176637659	A	-	0.357142		
TCGA-CN-A6V3-01A-12D-A34J-08	p.C1710Y	Missense	176687152	176687152	G	A	0.096774	High	
TCGA-CN-A6V3-01A-12D-A34J-08	p.W1769C	Missense	176696606	176696606	G	T	0.45	Medium	
TCGA-UF-A71D-01A-12D-A34J-08	p.T922fs	Frame_Shift_Del	176638165	176638165	C	-	0.433962		
TCGA-BA-A6DE-01A-22D-A31L-08	p.E1575X	Nonsense	176678812	176678812	G	T	0.47619		
TCGA-CV-A6JU-01A-11D-A31L-08	p.Y1941fs	Frameshift Insertion	176707765	176707765	-	T	0.232143		
TCGA-QK-A6VB-01A-12D-A34J-08	p.Y1834fs	Frameshift Insertion	176696801	176696801	-	A	0.659091		
TCGA-P3-A6T8-01A-11D-A34J-08	p.S2664fs	Frame_Shift_Del	176722361	176722361	T	-	0.46		
TCGA-UF-A719-01A-12D-A34J-08	p.S1528Y	Missense	176675267	176675267	C	A	0.7	Low	Sample also has H3K36M mutation
TCGA-UF-A7JF-01A-11D-A34J-08	p.E1853X	Nonsense	176700720	176700720	G	T	0.494118		
TCGA-QK-A8Z8-01A-11D-A391-08	p.H1872fs	Frame_Shift_Del	176700778	176700779	AT	-	0.708333		

Supplementary Table 2. Complex genomic events in H3K36 samples with no NSD1 or H3K36M mutations by Whole Exome Sequencing. LOH=Loss Of Heterozygosity; NA=Not Available

Sample	Result	GISTIC NSD1 Gene Value	GISTIC NSD1 Gene Threshold
CV-7091	LOH	-0.36	-1
CN-4731	Focal Deletion of 8 exons	-1.243	-2
D6-6823	Splicing defect	-0.508	-1
CN-6988	LOH	-0.563	-1
BA-4074	No expression	NA	NA
CV-7428	Not sequenced	-0.002	0
CV-7435	No expression	-1.047	-2
UF-A7JH	Focal deletion exons 4-18	-1.295	-2

Supplementary Table 3. NSD1 mutations in samples excluded from the H3K36 cluster. Hugo Symbol NSD1, Chromosome 5.

Sample ID	Protein Change	Mutation Type	Start Position	End Position	Reference Allele	Variant Allele	Allele Frequency	Mutation Assessor
TCGA-CN-4739-01A-02D-1512-08	p.C1710S	Missense	176687152	176687152	G	C	0.291667	High
TCGA-HD-7917-01A-11D-2229-08	p.E1202fs	Frame_Shift_Del	176639004	176639004	G	-	NA	
TCGA-DQ-7595-01A-11D-2229-08	p.P803fs	Frame_Shift_Del	176637807	176637807	C	-	NA	
TCGA-CR-6481-01A-11D-1870-08	p.D1489N	Missense	176673765	176673765	G	A	0.185714	Neutral
TCGA-CR-7395-01A-11D-2012-08	p.L2054R	Missense	176715829	176715829	T	G	NA	High
TCGA-CV-6942-01A-21D-2012-08	p.E2467Q	Missense	176721768	176721768	G	C	0.164384	Low
TCGA-CV-6956-01A-21D-2012-08	p.R1984X	Nonsense	176709523	176709523	C	T	0.578125	
TCGA-CR-7385-01A-11D-2012-08	p.R1700X	Nonsense	176687121	176687121	C	T	0.072463	
TCGA-CV-7427-01A-11D-2078-08	p.D1992H	Missense	176709547	176709547	G	C	0.261261	Neutral
TCGA-CV-7427-01A-11D-2078-08	p.D2002H	Missense	176709577	176709577	G	C	0.258427	Medium
TCGA-CN-5360-01A-01D-1434-08	p.S744X	Nonsense	176637631	176637631	C	G	0.308642	
TCGA-CV-6003-01A-11D-1683-08	p.I2113M	Missense	176719035	176719035	C	G	0.111111	Low
TCGA-CV-6441-01A-11D-1683-08	p.D325V	Missense	176618931	176618931	A	T	0.724138	Medium
TCGA-CV-6961-01A-21D-1912-08	p.S96C	Missense	176562391	176562391	C	G	0.206897	Neutral
TCGA-CV-7568-01A-11D-2229-08	p.P434L	Missense	176636701	176636701	C	T	0.40625	Low
TCGA-CV-7568-01A-11D-2229-08	p.P434S	Missense	176636700	176636700	C	T	0.412698	Low
TCGA-D6-8569-01A-11D-2394-08	p.V366L	Missense	176631153	176631153	G	T	0.279221	Low
TCGA-CV-A468-01A-11D-A25Y-08	p.R1320X	Nonsense	176665274	176665274	C	T	0.067961	
TCGA-CV-A45Z-01A-21D-A25D-08	p.K601X	Nonsense	176637201	176637201	A	T	0.173077	
TCGA-F7-A623-01A-11D-A28R-08	p.E1520K	Missense	176675242	176675242	G	A	0.116279	Low
TCGA-UF-A71B-01A-12D-A34J-08	p.G566E	Missense	176637097	176637097	G	A	0.139785	Neutral
TCGA-TN-A7HL-01A-11D-A34J-08	p.R1634Q	Missense	176684087	176684087	G	A	0.242424	Medium
TCGA-P3-A6T6-01A-11D-A34J-08	p.E1516X	Nonsense	176675230	176675230	G	T	0.636364	
TCGA-QK-AA3J-01A-11D-A391-08	p.R788X	Nonsense	176637762	176637762	C	T	0.71875	

Supplementary Table 4. H3K36M Mutations in TCGA HNSCCs

Hugo Symbol	Sample ID	Protein Change	Mutation Type	Chromosome	Start Position	End Position	Reference Allele	Variant Allele	Allele Frequency
H3F3B	TCGA-CQ-6228-01A-11D-1912-08	p.K37M*	nonsynonymous SNV	17	73775146	73775146	T	A	0.416667
H3F3B	TCGA-QK-A8Z7-01A-11D-A391-08	p.K37M	nonsynonymous SNV	17	73775146	73775146	T	A	0.552941
HIST1H3C	TCGA-CN-4740-01A-01D-1434-08	p.K37M	nonsynonymous SNV	6	26045748	26045748	A	T	0.163636
HIST1H3C	TCGA-CQ-6225-01A-11D-1912-08	p.K37M	nonsynonymous SNV	6	26045748	26045748	A	T	0.33871
HIST1H3C	TCGA-CQ-A4C9-01A-11D-A25D-08	p.K37M	nonsynonymous SNV	6	26045748	26045748	A	T	0.315789
HIST1H3C	TCGA-MT-A67D-01A-31D-A30E-08	p.K37M	nonsynonymous SNV	6	26045748	26045748	A	T	0.20202
HIST1H3E	TCGA-CV-6954-01A-11D-1912-08	p.K37M	nonsynonymous SNV	6	26225492	26225492	A	T	0.619354
HIST1H3G	TCGA-UF-A7JD-01A-11D-A34J-08	p.K37M	nonsynonymous SNV	6	26271503	26271503	T	A	0.227941
HIST1H3I	TCGA-CV-7263-01A-11D-2012-08	p.K37M	nonsynonymous SNV	6	27839984	27839984	T	A	0.197674
HIST1H3I	TCGA-UF-A719-01A-12D-A34J-08	p.K37M	nonsynonymous SNV	6	27839984	27839984	T	A	0.281481
HIST2H3D	TCGA-CX-7086-01A-11D-2078-08	p.K37M	nonsynonymous SNV	1	149785127	149785127	T	A	0.125

*K37M is the universal nomenclature when describing amino acid changes in genomic data. We note that histone proteins have the first methionine removed from the final product thus shifting the numbering by one amino acid. In the context of protein sequences, this mutation will generally be referred to as K36M.

Supplementary Table 6. Meta analysis of previous HNSCC sequencing studies reveals other NSD1 and H3K36M mutations. Table adapted from *Riaz, Morris, Lee, & Chan. Unraveling the molecular genetics of head and neck cancer through genome-wide approaches. Genes Dis **1;** 75-86, 2014*

Sequencing Study (# Samples Sequenced)	NSD1	H3K36M
Stransky (74)	p.C1710* (Larynx, Smoker, HPV-), p.C2124Y (Larynx, Smoker, HPV-), p.P1726H (Larynx, Smoker, HPV-), p.R1233fs (Larynx, Smoker, HPV-), p.R1984Q (Hypopharynx, Smoker, HPV-), p.Y1834fs (Larynx, Smoker, HPV-), splicing (Oral Cavity, Smoker, HPV-)	HIST1H3H (Oral Cavity, Smoker, HPV-)
Agrawal (32)	0	0
Pickering (40)	p.R2017Q (Tongue, Smoker, HPV-), p.W2032R (FOM, Smoker, HPV-), p.R1984X (FOM, Smoker, HPV-)	0
India ICGC (50)	p.Q517* (Gingivo-buccal oral , NA, NA) , p.S958L (Gingivo-buccal oral , NA, NA), p.R1811* (Gingivo-buccal oral , NA, NA), p.C1911F (Gingivo-buccal oral , NA, NA)	0