

Supplementary information

The file contains

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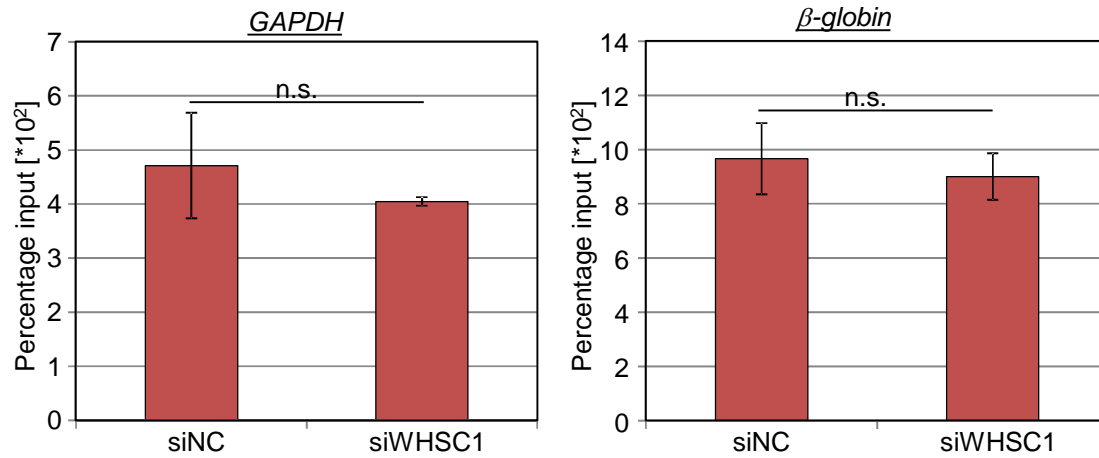


Figure S1. Chromatin immunoprecipitation assays (ChIP) performed in UD-SCC-2 cells treated with siWHSC1 for 3 days. Real-time PCR analysis was conducted using primer pairs as described under “Materials and Methods”. Immunoprecipitation was performed with ChIP grade anti-H3K36me2 (Millipore, 07-369) antibodies. Y-axis shows a percentage of the input chromatin. Results are the mean \pm SD of three independent experiments, and *P*-values were calculated using Student’s *t*-test (n.s.: not significant).

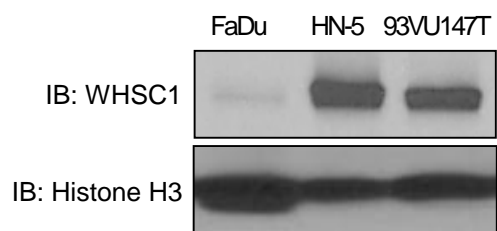


Figure S2. WHSC1 protein expression of three SCCHN (FaDu, HN-5 and 93VU147T) cells. Nuclear extracts were immunoblotted with anti-WHSC1 (Abcam, ab75359) and anti-histone H3 (Abcam, ab1791) antibodies. Histone H3 was served as an internal control.

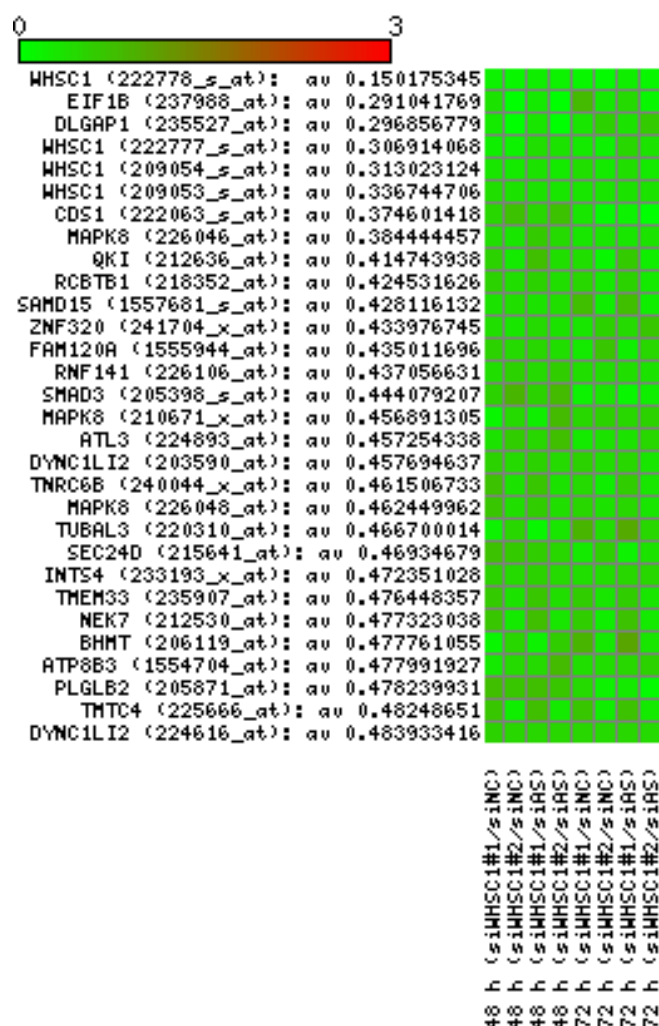


Figure S3. Heatmap of down regulated-genes at 48 h and 72 h of WHSC1 knockdown in UD-SCC-2 cells.

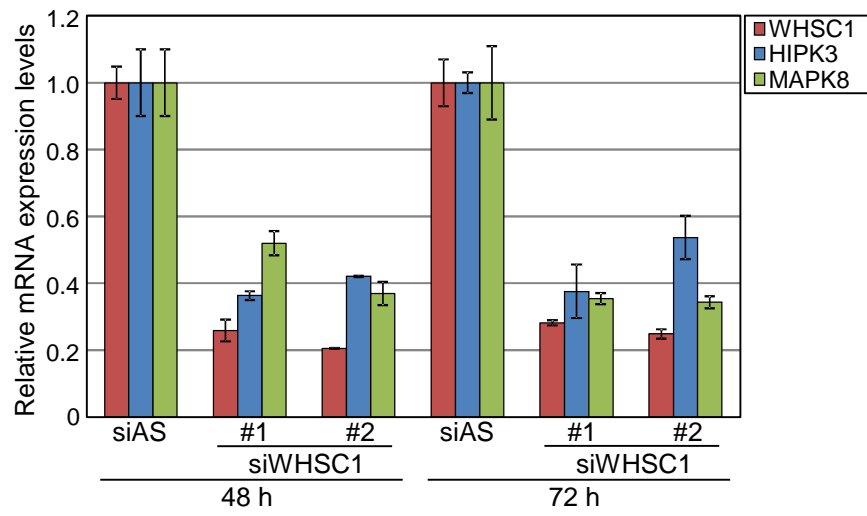


Figure S4. Validation of *WHSC1*, *HIK3* and *MAPK8* expressions in HPV-positive (UD-SCC-2) cells by quantitative real-time PCR 48 or 72 h after treatment with siRNAs.

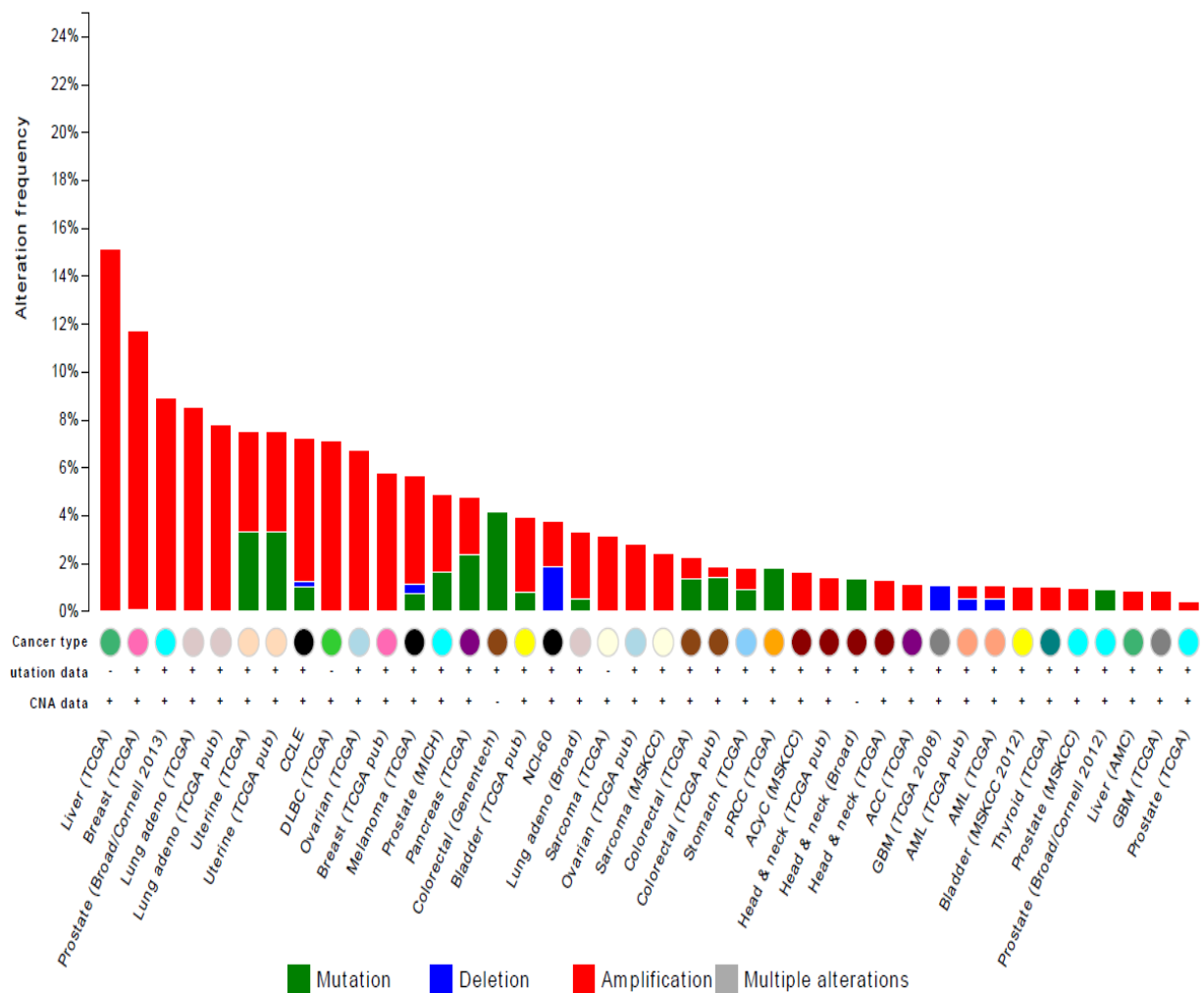


Figure S5. Cross-cancer alteration summary for NEK7. The data were obtained from the TCGA database (<http://www.cbioportal.org/public-portal/>).

Table S1. Characteristics of SCCHN cell lines.

Cell name	TNM stage	Specimen site	Gender	HPV status
UD-SCC-2	T1N2M0	Hypopharynx	Male	HPV16-positive
UM-SCC-23	T3N0M0	Larynx	Female	HPV16-negative
SCC-25	T2N1M0	Oral cavity	Male	HPV16-negative
SCC-35	T4N0M0	Hypopharynx	Male	HPV16-negative
UT-SCC-40	T3N0M0	Oral cavity	Male	HPV16-negative
HN-SCC-135	T1N1M0	Hard Palate	-	HPV16-negative
HN-SCC-151	T3N0M0	Oral tongue	-	HPV16-negative
HN-SCC-166	T4N0M0	Hard Palate	-	HPV41-positive
PE/CA-PJ15	-	Oral tongue	Male	HPV16-negative
OECM-1	-	Oral cavity	Male	HPV16-negative
BICR31	T4N2M0	Oral tongue	Male	HPV16-negative
93VU147T	T4N2M0	Floor of mouth	Male	HPV16-positive
FaDu	-	Hypopharynx	Male	HPV16-negative
JSQ-3	T3N0M0	Nasal vestibule	Male	HPV16-negative
HN-5	T2N0M0	Oral cavity	Male	HPV16-negative
HN-6	T2N0M0	Oral cavity	Male	HPV16-negative

Table S2. Primer Sequences for Quantitative RT-PCR.

Gene name	Primer sequence
<i>GAPDH</i> (housekeeping gene)-f	5' GCAAATTCCATGGCACCGTC 3'
<i>GAPDH</i> (housekeeping gene)-r	5' TCGCCCCACTTGATTTTGG 3'
<i>SDH</i> (housekeeping gene)-f	5' TGGGAACAAGAGGGCATCTG 3'
<i>SDH</i> (housekeeping gene)-r	5' CCACCACTGCATCAAATTCATG 3'
<i>WHSC1</i> -f	5' AATATGACTCCTTGCTGGAGCAGG 3'
<i>WHSC1</i> -r	5' ATTTCAACAGGTGGTCTTTGTCTC 3'
<i>HIPK3</i> - f	5' AAG AGC ATG AGG CAG AGA CAG 3'
<i>HIPK3</i> -r	5' TCC TTC CAA ATC CAT CAC TGT G 3'
<i>MAPK8</i> -f	5' GAA GCT CCA CCA CCA AAG ATC 3'
<i>MAPK8</i> -r	5' TTG GTT CTC TCC TCC AAG TCC 3'
<i>NEK7</i> -f	5' CAC CTG TTC CTC AGT TCC AAC 3'
<i>NEK7</i> -r	5' CTC CAT CCA AGA GAC AGG CTG 3'

Table S3. siRNA Sequences.

siRNA name	Sequence	
siNegative control (Cocktail)	Target#1	Sense: 5' AUCCGCGCGAUAGUACGUA 3'
		Antisense: 5' UACGUACUAUCGCGCGGAU 3'
	Target#2	Sense: 5' UUACGCGUAGCGUAAUACG 3'
		Antisense: 5' CGUAUUACGCUACGCGUAA 3'
	Target#3	Sense: 5' UAUUCGCGCGUAUAGCGGU 3'
		Antisense: 5' ACCGCUAUACGCGCGAAUA 3'
siWHSC1#1		Sense: 5' CAGAUCUACACAGCGGAUA 3'
		Antisense: 5' UAUCCGCUGUGUAGAUCUG 3'
siWHSC1#2		Sense: 5' GUUAAUUGGCAUAUGGAAU 3'
		Antisense: 5' AUUCCAUAUGCCAAUUAAC 3'

Table S4. Gene Ontology analysis of candidate genes.

Gene Ontology	P-value	Genes
Downregulated $\geq 50\%$ baseline (26 genes)		
Translation	<0.05	EIF1B, TNRC6B
Transcription Regulation		RCBTB1, ZNF320, RNF141, SMAD3, HIPK3
snRNA Processing		INTS4
mRNA Processing		QKI
Cytokinesis		NEK7
MAPK/JNK Cascade		MAPK8
Apoptosis		HIPK3, MAPK8
Fibroblast-growth-factor receptor		TNRC6B
Signaling		MAPK8
Neuronal Differentiation		MAPK8
Postembryonic Development		STX2
Epithelial/Cellular Differentiation		CDS1
Phosphatidylinositol		ATL3, TUBAL3, BHMT, ATP8B3
Biosynthetic Process		DLGAP1
Metabolic Processes		DYNC1LI2
Cell-cell Signaling		SEC24D
Cytoplasmic Transport		
Antigen Processing		
Upregulated ≥ 2-fold baseline (5 genes)		
Protein Phosphorylation	<0.05	DAPK1
TOR Signaling		PIK3AP1
Cell Differentiation		PPDPF
Metabolic Processes		UGT2A1/2
Axonogenesis		SLITRK6