SUPPLEMENTARY TABLES

Supplementary Table S1. HNSCC *NOTCH* mutation samples in TCGA

Supplementary Table S2. *NOTCH4* and *HEY1* high/low group clinical demographics in TCGA

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure S1. The correlation of *NOTCH* pathway genes and the *HES/HEY* high group.

(A) Table (left) shows a comparison of *NOTCH* pathway gene expression in HNSCC tumors with *HES* or *HEY* activation. The *HES/HEY* high group is defined as tumors with expression 1 standard deviation greater than the mean of normal tissue for *HES/HEY*. Ratio is calculated by dividing the mRNA expression of the high group by the expression of the low group. The heat map (right) depicts the gene expression of these genes in tumors of the *HEY* high and low groups. Increased expression is presented in green, and decreased expression is presented in red.

Supplementary Figure S2. *NOTCH4* expression in si-*NOTCH4* cells. *NOTCH* activity assay, cisplatin viability assay, apoptosis assay and cell cycle analysis in si-*NOTCH4* cells.

(A) *NOTCH4* expression of parental, si-control and si-*NOTCH4* cells. mRNA expression is measured by qRT-PCR. The expression differences between si-control and si-*NOTCH4* cells are compared. *P* value is calculated by using Student's *t*-test. **: $P < 0.01$. (B) Western blot analysis of *NOTCH4* and *GAPDH* in si-control and si-*NOTCH4* cells. (C) Representative images of *NOTCH* activity assay in SCC090 si-control and si-*NOTCH4* cells. light grey shadow shows the negative control cells. (D) Cisplatin viability assay. Cisplatin concentration ranges from 0.1 μM to 81 μM. Cell viability is measured after exposed to cisplatin for 3 days. (E) Flow cytometry images of apoptosis assay in SCC61 si-control and si-*NOTCH4* cells. The lower right box (FITC+, PI-) means the early apoptotic cell area. The upper right (FITC+, PI+) box means the late apoptotic cell area. (F) Flow cytometry images of cell cycle analysis in SCC090 si-control and si-*NOTCH4*. The blue area means the cell cycle phase distribution of

si-*NOTCH4* cells. The red area means that of si-control cells.

Supplementary Figure S3. Sphere colony shapes. ***HEY1*** **expression in si-*HEY1* cells and *NOTCH4* expressions in sh-*NOTCH4* cells**

(A) Sphere colony shapes of the HNSCC cell lines. Scale bar indicates 10 μm. (B) *HEY1* expression of parental, si-control and si-*HEY1* cells. mRNA expression is measured by qRT-PCR. The expression differences between si-control and si-*HEY1* cells are compared. (C) Western blots of *HEY1* and *GAPDH* in si-control and si-*HEY1* cells. (D) Transfection efficiency of the sh-control and sh-*NOTCH4* vector in Cal27. (E) *NOTCH4* expression of sh-control and sh-*NOTCH4* Cal27 cells. The expression differences between sh-control and sh-*NOTCH4* cells are compared. *P* value is calculated by using Student's *t*-test. **: $P < 0.01$. (F) Western blots of *NOTCH4* and *GAPDH* in sh-control and sh-*NOTCH4* Cal27 cells.

Supplementary Figure S4. **Comparison of EMT-related genes between *HEY1* high and low groups using TCGA data**

The high and low groups are divided by the average of HNSCC *HEY1* expression. The boxes represent the interquartile range (25^{th}-75^{th}), and horizontal lines inside the boxes indicate median. Whiskers indicate the minimum and maximum values. *P* value is calculated by using Student's *t*-test. Ratio is calculated by dividing the mRNA expression of the high group by the expression of the low group.

Supplementary Figure S5. **Comparison of HNSCC CSCs marker genes between *NOTCH4, HEY1* high and low groups using the TCGA data set. *ALDH1* expression in si-*NOTCH4* and si-*HEY1* cells.**

(A, B) The boxes represent the interquartile range (25^{th}-75^{th}), and horizontal lines inside the boxes indicate median. Whiskers indicate the minimum and maximum values. Ratio is calculated by dividing the mRNA expression of the high group by the expression of the low group. (C) *ALDH1* expression of parental, si-control si-*NOTCH4* and si-*HEY1* cells. mRNA expression is measured by qRT-PCR. The expression differences between si-control and si-*NOTCH4*/si-*HEY1* cells are compared. (D) Flow cytometry images of aldefluor assay in SKN3 si-*NOTCH4* and si-*HEY1* cells. Diethylaminobenzaldehyde (DEAB) was used to inhibit *ALDH1* activity. The gate (bold line region) for *ALDH1* +

cells is determined in relation to the DEAB control (DEAB+). (E) The fraction of *ALDH1* positive cells in parental, si-control, si-*NOTCH4* and si-*HEY1* cells. The *ALDH1* + cells expression differences between si-control and si-*NOTCH4/si-HEY1* cells are compared. *P* value is calculated by using Student's *t*-test. **: $P < 0.01$.

Supplementary Figure S6. **Comparison of overall survival between *NOTCH4, HEY1* high and low groups using the TCGA data set.** (A) Overall survival in *NOTCH4* high and low group (B) Overall survival in *HEY1* high and low group. These high and low group is divided by the mean expression of tumor samples. *P* value is calculated by using Log-Rank test.