

## Supplementary figure legends

**Supplementary Figure 1. Significant overexpression of *JAG1* (A) and *PSEN1* (B) in the tumors with increased copy number gains in comparison to that in the normal tissues in the cohort of 44 HNSCC tumors and 25 normal mucosa.** N, normal mucosa; T, HNSCC tumors; -, without *JAG1*(A) or *PSEN1* (B) copy number gain; +, with *JAG1* (A) or *PSEN1* (B) copy number gain. Boxes represent the interquartile range (25<sup>th</sup>-75<sup>th</sup> percentile) and horizontal lines inside the boxes indicate median. Whiskers indicate the minimum and maximum values. P value was calculated by using t test.

**Supplementary Figure 2. NOTCH pathway activation in HNSCC. A, Immunohistochemical analysis of NOTCH1, HES1 and HEY1 protein expression in HNSCC.** Tissue microarray arrays with quadruplicates of samples from each of 56 HNSCC tumor and 11 non-tumor patients were stained with anti-NOTCH1, anti-HES1 or anti-HEY1 antibody, as described in methods. Top, the heat-map of immunohistochemical analysis for tissue microarray slides. Rows, genes; column, each individual sample. The average staining for four tissues was categorized to Strong (brown), Moderate (grey) or Weak/Negative (blue) staining. Samples are ranked by the pathology ID numbers in tumor (left) and normal (right) groups. Bottom: quantification of immunohistochemical data. The number and percentage of tumor (Tu) and normal (No) patient in strong, moderate and weak/negative category was quantified. P-values were calculated by comparing numeric scores in tumor vs normal sample by independent t test. The samples 57964, 58602, 22146, 5931, 26514 represent tumor samples X6, X7, X11, X28 and X34 from the original discovery cohort of 44 HNSCC tumors. **B, Representative immunohistochemical results.** The representative slides of positive or moderate staining are shown for tumors and compared to normal tissue. Bar, 100 um equivalent. Tumor (T) and normal (N) tissues on each sample are labeled. ID 22146 represented X11 samples, which were used both in array and in immunohistochemical analysis, where protein and RNA expression strongly correlate.

**Supplementary Figure 3. Validation of *JAG1*, *JAG2*, *NOTCH1* and *PSEN1* overexpression in TCGA HNSCC cohort. A, Overexpression of *JAG1*, *JAG2* and *NOTCH1* in tumors in comparison to that in normal tissues in the TCGA HNSCC cohort. B, Overexpression of *JAG1* and *PSEN1* in the tumors with increased copy number gains in comparison to that in the normal tissues as well as to that in the tumors without copy number gains in the TCGA HNSCC cohort.** N, normal mucosa; T, HNSCC tumors; -, without *JAG1* or *PSEN1* copy number gain; +, with *JAG1* or *PSEN1* copy number gain. Dots, relative expression levels in different tissue samples. Boxes represent the interquartile range (25<sup>th</sup>-75<sup>th</sup> percentile) and horizontal lines inside the boxes indicate median. Whiskers indicate the minimum and maximum values. P value was calculated by using t test.

**Supplementary Figure 4. Growth effects of *NOTCH1* siRNA inhibition in HNSCC cells with wildtype *NOTCH1* status.** *NOTCH1* was downregulated in UPCI-SCC090 (090, top, *NOTCH1* wt) and SCC61 (bottom, *NOTCH1* wt) by siRNA as described in methods. The effect of *NOTCH1* downregulation on cell proliferation for these two cell lines is shown in the middle. The effect of si*NOTCH1* on *NOTCH1* expression (left) or expression of its targets (*HES1* and *HEY1*, right) is also shown. The mRNA expression levels were calculated relative to *GAPDH* and were normalized to 1 in the control experiments with scrambled siRNA sequences. Experiments were performed in pentaplicates, mean  $\pm$  SEM were shown. \*, p-value < 0.05.

**Supplementary Figure 5. Growth effects of *HEY1* siRNA inhibition in HNSCC cells with wildtype *NOTCH1* status.** *HEY1* was downregulated in UPCI-SCC090 and SCC61 by siRNA as described in methods. The effect of si*HEY1* on the expression of *HEY1* is shown on the left. The effect of *HEY1* downregulation on cell proliferation for these two cell lines is shown on the right. Note, there is over 90% transfection efficiency of *HEY1* downregulation for each cell line, however the strong growth inhibition effect was shown only for *NOTCH1* wildtype 090 cell line.

**Supplementary Figure 6. Effect of  $\gamma$ -secretase inhibitor on the *NOTCH1* pathway genes and cell growth in HNSCC cells with *NOTCH1* wildtype status.** UPCI-SCC090 cells were growing on the media supplemented with 0.2  $\mu$ M  $\gamma$ -secretase inhibitor XXI (GSI-XXI) for up to 72 hours. The effect of GSI-XXI on the *NOTCH1* targets *HES1* and *HEY1* is shown on the left for UPCI-SCC090 cell line.