

Figure S1. *HEY1* expression, migration and invasion assays of *HEY1*-OE cells. (A) Analyses of *HEY1* expression in *HEY1*-OE and control Cal27 cells by RT-qPCR. *GAPDH* antibody was used as a control. (B) Representative images of migration and invasion assays. The cell line used here was SCC090. Scale bar, 100  $\mu$ m. \*\* $P < 0.01$ . *HEY1*-OE cells, *HEY1*-overexpressing cells.

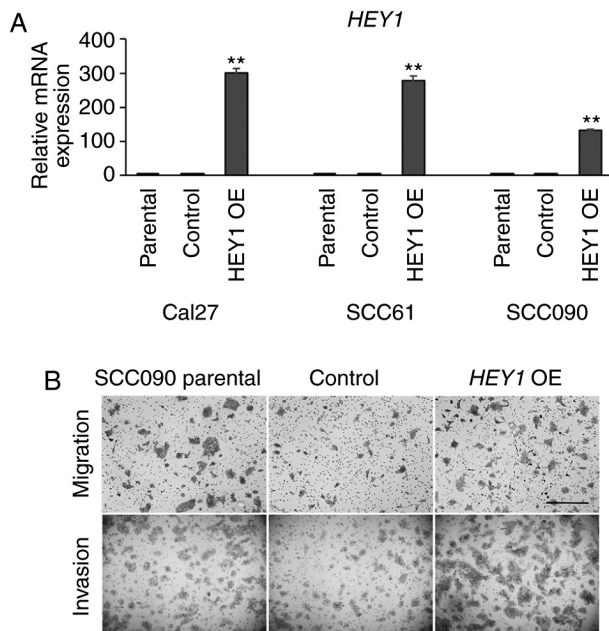


Figure S2. Proliferation assay and mouse xenograft tumors sizes with *HEY1*-OE cells. (A) Proliferation assays. Cell growth of *HEY1*-OE cells is compared to control cells on day 3. P-values were calculated using Dunnett's t-test. \*\*P<0.01. (B) Images of tumors harvested from nude mice. These mice were injected with  $2 \times 10^6$  cells of *HEY1*-OE and control Cal27 cells into their flanks. *HEY1*-OE cells, HEY1-overexpressing cells.

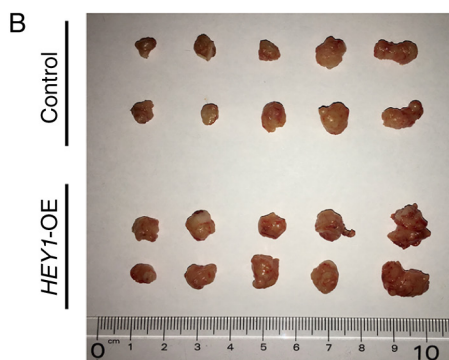
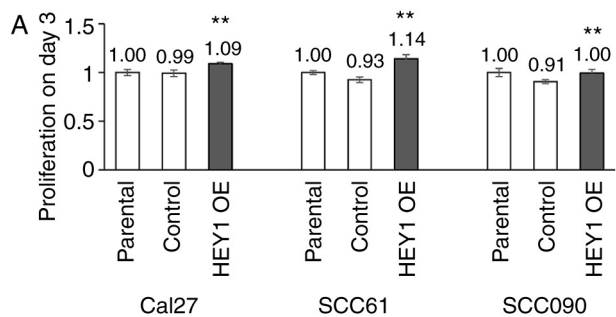


Figure S3. Correlations between *HES1*, *HES5*, *HEY1* and *SOX2* expression in the TCGA HNSCC data set. *SOX2* expression in *SOX2*-OE cells. (A) Correlations between *SOX2* and *HES1* and 5 were examined using the TCGA dataset, including HNSCC (n=522). (B) Correlations between *SOX2* and *HEY1* are examined including HPV-negative (n=421) and -positive HNSCC (n=97). 'r' indicates the Pearson's correlation coefficient. (C) *SOX2* expression was compared between *SOX2*-OE and control cells by RT-qPCR. P-values were calculated using Dunnett's t-test. \*\*P<0.01. *SOX2*-OE cells, *SOX2*-overexpressing cells.

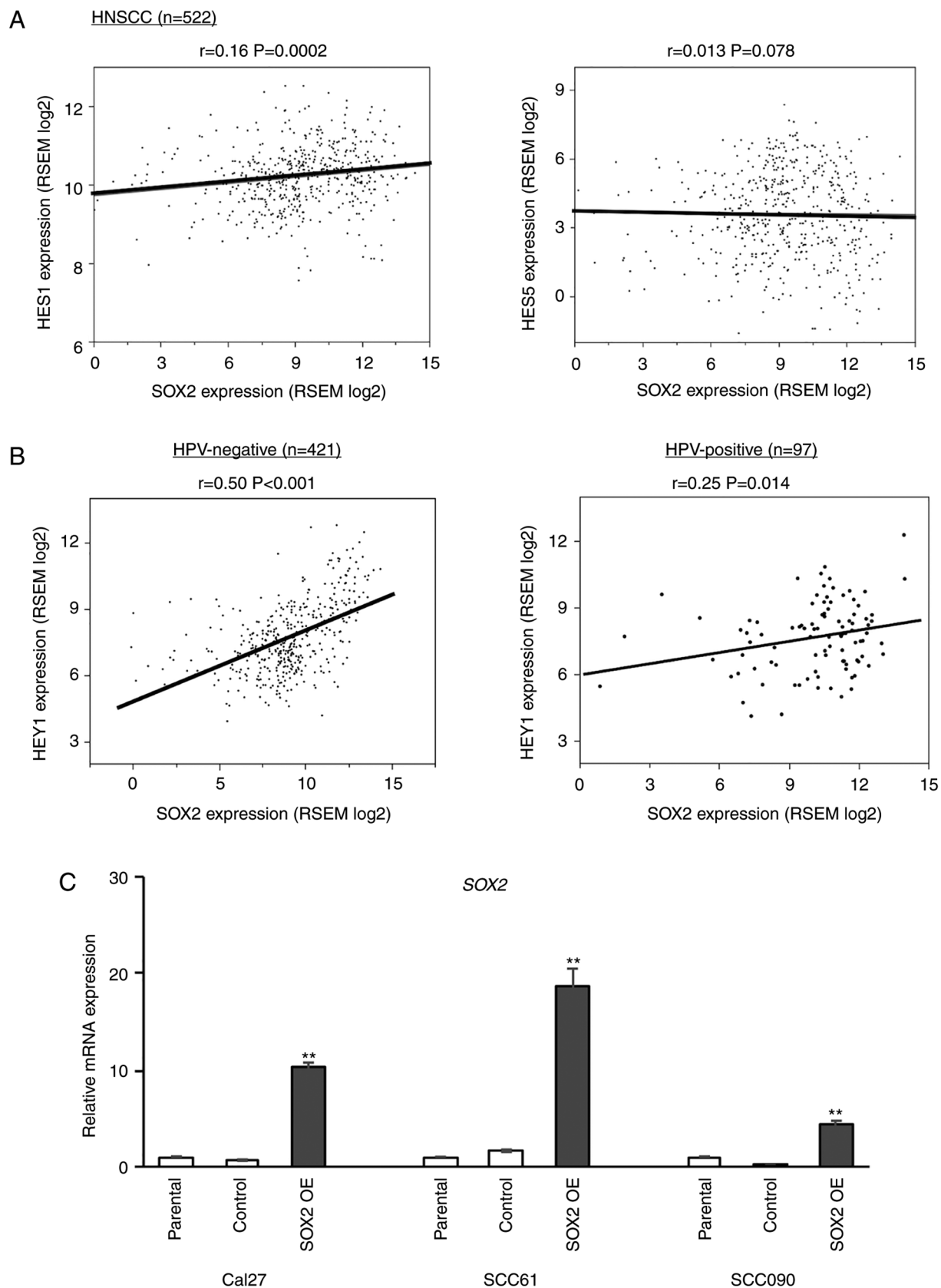


Figure S4. Correlations between *HEY1* and *NOTCH1*, 2, 3, and 4 expression in the TCGA HNSCC dataset and the correlation between *HEY1*, *HES1*, *HES5* and *NOTCH4* expression in the TCGA normal samples dataset. (A) Correlations between *NOTCH4* and *HEY1* were examined in HPV-negative (n=421) and -positive HNSCC (n=97). (B) Correlations between *NOTCH1-3* and *HEY1* were examined using the TCGA HNSCC dataset, including *NOTCH* wild-type HNSCC (n=447). HNSCC samples with any *NOTCH* mutation were excluded (n=73). (C) Western blot analysis of the EMT-related protein, N-cadherin, in control and *HEY1*-OE cells. GAPDH was used as a control. (D) Correlations between *NOTCH4* and *HEY1*, *HES1*, and *HES5* were examined using the TCGA head and neck normal sample dataset (n=44). ‘r’ indicates the Pearson's correlation coefficient. *HEY1*-OE cells, *HEY1*-overexpressing cells.

