Figure S1 Misawa K

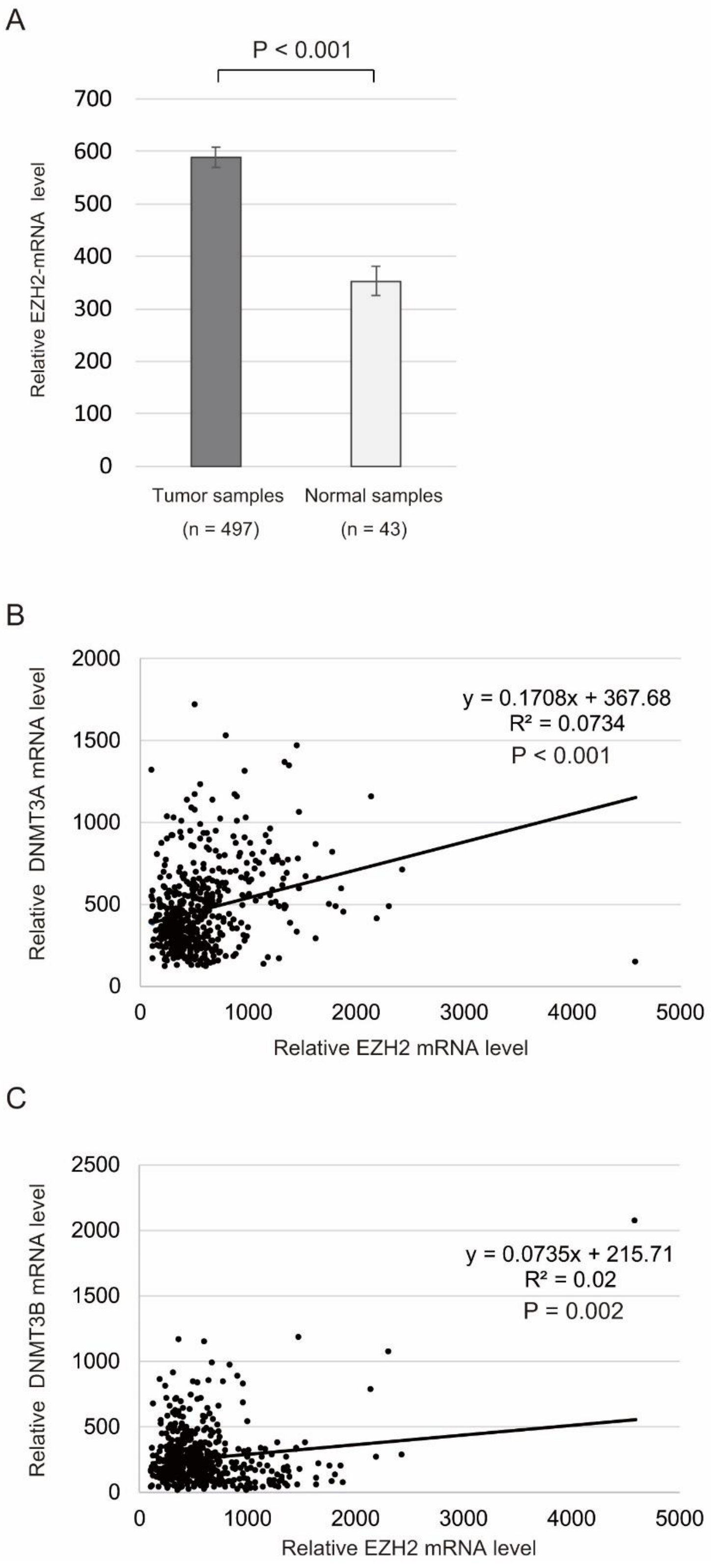

**Figure S1.** Data regarding EZH2, DNMT3A, and DNMT3B mRNA expression obtained from The Cancer Genome Atlas (TCGA). Data regarding *EZH2*, *DNMT3A*, and *DNMT3B* mRNA expression in head and neck squamous cell carcinoma were obtained from TCGA (https://tcga-data.nci.nih.gov/tcga/) and MethHC (http://methhc.mbc.nctu.edu.tw/php/index.php).

**Figure S2 Misawa K**

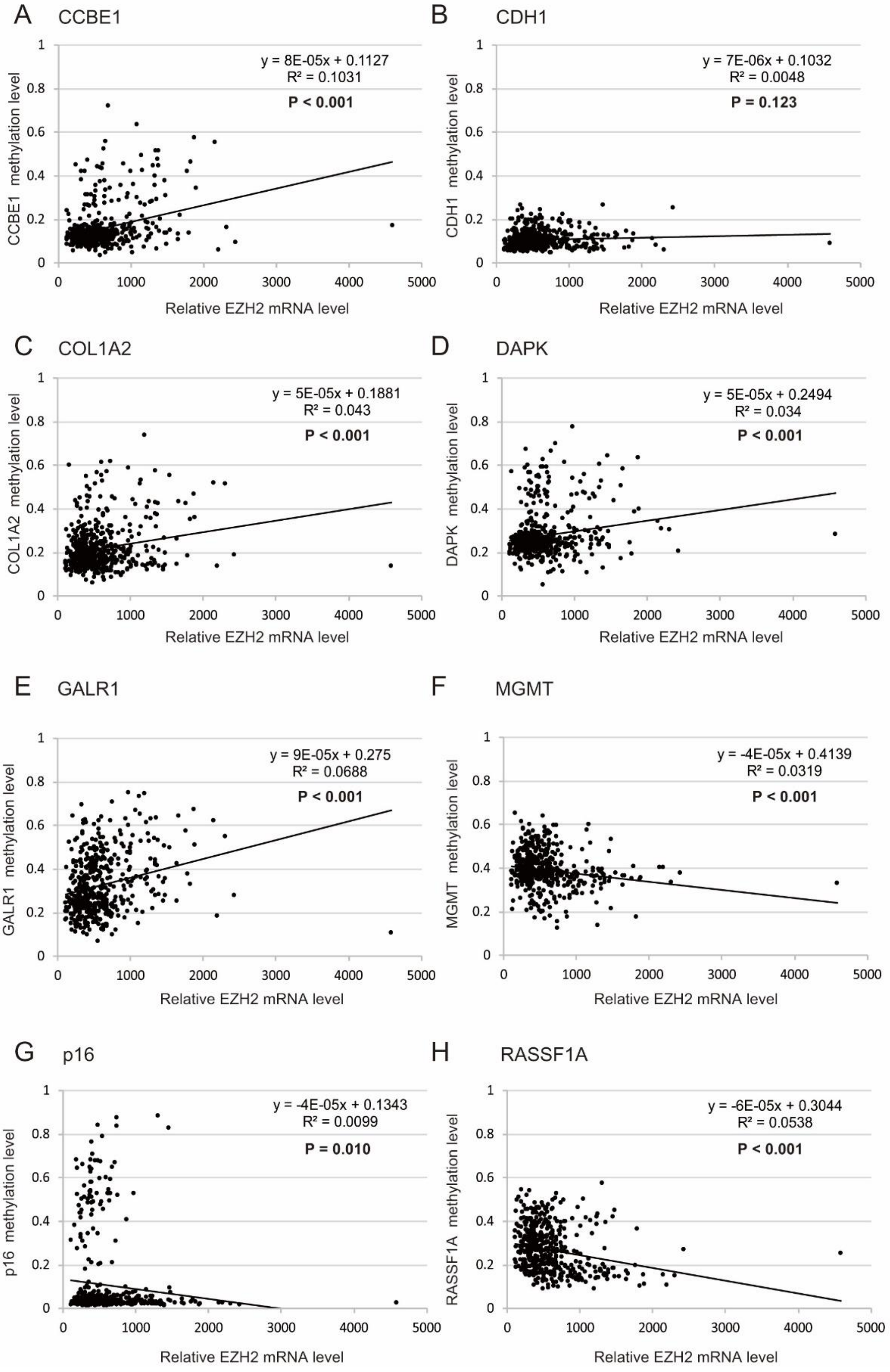

**Figure S2 Misawa K**

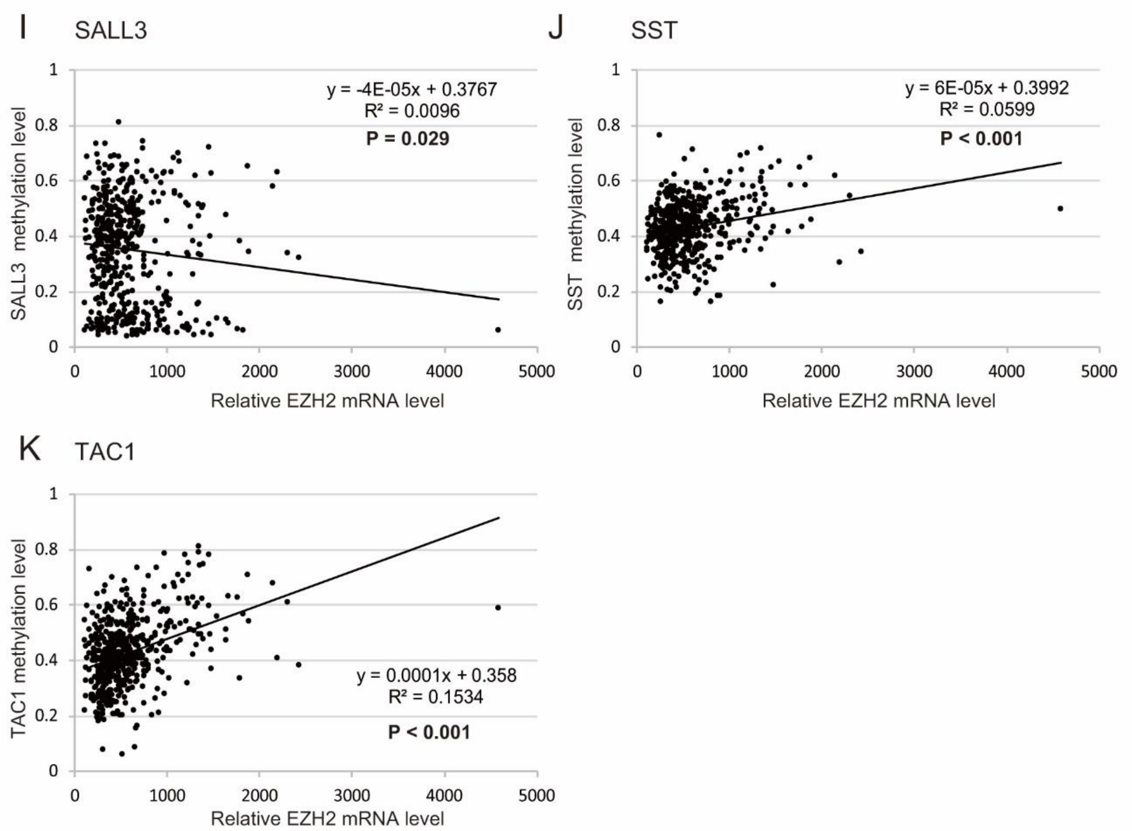

**Figure S2.** Data regarding DNA methylation obtained from The Cancer Genome Atlas (TCGA).

**Table S1.** Baseline Characteristics of the 230 Patients.

| Patient and tumor characteristics | Original cohort (n = 110) | Validation cohort (n = 120) | Original and Validation cohorts (n = 230) |
|---|---|---|---|
| *Age* | | | |
| Under 70 | 82 (74.5%) | 75 (62.5%) | 157 (68.3%) |
| 70 and older | 28 (25.5%) | 45 (37.5%) | 73 (31.7%) |
| *Gender* | | | |
| Male | 87 (79.1%) | 103 (85.8%) | 190 (82.6%) |
| Female | 23 (20.9%) | 17 (14.2%) | 40 (17.4%) |
| *Alcohol exposure* | | | |
| Ever | 60 (54.5%) | 99 (82.5%) | 159 (69.1%) |
| Never | 50 (45.5%) | 21 (17.5%) | 71 (30.9%) |
| *Smoking status* | | | |
| Smoker | 72 (65.5%) | 93 (77.5%) | 165 (71.7%) |
| Non-smoker | 38 (34.5%) | 27 (22.5%) | 65 (28.3%) |
| *Tumor size* | | | |
| T1 | 11 (10.0%) | 12 (10.0%) | 23 (10.0%) |
| T2 | 46 (41.8%) | 37 (30.8%) | 83 (36.1%) |
| T3 | 21 (19.1%) | 26 (21.7%) | 47 (20.4%) |
| T4 | 32 (29.1%) | 45 (37.5%) | 77 (33.5%) |

| | | | |
|---|---|---|---|
| *Lympho-node status* | | | |
| N0 | 51 (46.4%) | 55 (45.8%) | 106 (46.1%) |
| N+ | 59 (53.6%) | 65 (54.2%) | 124 (53.9%) |
| *Stage* | | | |
| I | 7 (6.4%) | 10 (8.3%) | 17 (7.4%) |
| II | 26 (23.6%) | 18 (15.0%) | 44 (19.1%) |
| III | 19 (17.3%) | 24 (20.0%) | 43 (18.7%) |
| IV | 58 (52.7%) | 68 (56.7%) | 126 (54.8%) |
| *Recurrence events* | | | |
| Positive | 56 (50.9%) | 40 (33.3%) | 96 (41.7%) |
| Negative | 54 (49.1%) | 80 (66.7%) | 134 (58.3%) |

**Table S2. Q-RT-PCR and Q-MSP primer list.**

| PCR | Gene | Forward primer 5’-3’ | Reverse primer 5’-3’ |
|---|---|---|---|
| Q-RT-PCR | EZH2 | CCCTGACCTCTGTCTTACTTGTGGA | ACGTCAGATGGTGCCAGCAATA |
| Q-RT-PCR | DNMT3A | AGTACGACGACGACGGCTA | CACACTCCACGCAAAAGCAC |
| Q-RT-PCR | DNMT3B | AGGGAAGACTCGATCCTCGTC | GTGTGTAGCTTAGCAGACTGG |
| Q-RT-PCR | GAPDH | GCACCGTCAAGGCTGAGAAC | TGGTGAAGACGCCAGTCTCTA |
| Q-MSP | CCBE1 | GTCGCGGAGGAGTAGGACGCTT | CTCGAAAACGACGACACCATC |
| Q-MSP | CDH1 | GTGGGCGGGTCGTTAGTTTC | ACCACAACCAATCAACGCGA |
| Q-MSP | COL1A2 | ACGGTAGTAGGAGGTTTCGG | CGCAAAACCCCTAAATCACCGACG |
| Q-MSP | DAPK | GGATAGTCGGATCGAGTTAACGTC | CCCTCCCAAACGCCGA |
| Q-MSP | GALR1 | GGTTCGCGGTATTCGGTAGT | GGTTCGCGGTATTCGGTAGT |
| Q-MSP | MGMT | TTCGACGTTCGTAGGTTTTCGC | GCACTCTTCCGAAAACGAAACG |
| Q-MSP | p16 | GTATTTTTTTCGAGTATTCGTTTACGGC | CAAATCCTCTAAAAAAACCGCGA |
| Q-MSP | RASSF1A | CGTTCGGTTCGCGTTTGTTAGC | TAACCCGATTAAACCCGTACTTCG |
| Q-MSP | SALL3 | GGGGTTCGAGCGTCGTTAGT | CCGTACTCGAAAACCCCGTC |
| Q-MSP | SST | GGGGCGTTTTTTAGTTTGACGT | AACAACGATAACTCCGAACCTCG |
| Q-MSP | TAC1 | GGCGGTTAATTAAATATTGAGCAGAAAGTCGC | AAATCCGAACGCGCTCTTTCG |
| Q-MSP | ACTB | TGGTGATGGAGGAGGTTTAGTAAGT | AACCAATAAAACCTACTCCTCCCTTAA |

**Table S3. EZH2 expression status with the methylation of other eleven genes.**

| Genes | Methylation status | High (N = 75) | Low (N = 141) | P-values† |
|---|---|---|---|---|
| CCBE1 | Methylated | 51 | 70 | |
| | Unmethylated | 24 | 71 | 0.010* |
| CDH1 | Methylated | 34 | 74 | |
| | Unmethylated | 41 | 67 | 0.322 |
| COL1A2 | Methylated | 35 | 45 | |
| | Unmethylated | 40 | 96 | 1 |
| DAPK | Methylated | 47 | 79 | |
| | Unmethylated | 28 | 62 | 0.386 |
| GALR1 | Methylated | 46 | 68 | |
| | Unmethylated | 29 | 73 | 0.086 |
| MGMT | Methylated | 29 | 52 | |
| | Unmethylated | 46 | 89 | 1 |
| p16 | Methylated | 27 | 48 | |
| | Unmethylated | 48 | 93 | 1 |
| RASSF1A | Methylated | 10 | 25 | |
| | Unmethylated | 65 | 116 | 0.444 |
| SALL3 | Methylated | 51 | 72 | |
| | Unmethylated | 24 | 69 | 0.021* |
| SST | Methylated | 66 | 116 | |
| | Unmethylated | 9 | 25 | 0.329 |
| TAC1 | Methylated | 52 | 85 | |
| | Unmethylated | 23 | 56 | 0.235 |

† Fisher’s exact probability test.

* P <0.05.