MicroRNA-31 regulates expression of *Wntless* in both *Drosophila melanogaster* and human oral cancer cells

Ji-Eun Jung¹,², Joo-Young Lee³, In-Ryoung Kim⁴, Sang-Mee Park⁴, Hye-Ryoun Park¹,²,³,⁴ and Ji-Hye Lee¹,²,³,⁴*

¹Department of Life Science in Dentistry, ²BK21Plus Project, ³Dental and Life Science Institute, ⁴Department of Oral Pathology, ⁵Department of Anatomy, School of Dentistry, Pusan National University, Yangsan 50612, Korea

*Corresponding Author:

Ji-Hye Lee

Department of Oral Pathology, School of Dentistry,

Pusan National University

49 Pusandaehak-Ro, Mulgeum-Eup

Yangsan-Si, Kyoungsangnam-Do 50612

Korea, Republic of

Phone: +82-51-510-8259

E-mail: jihyelee@pusan.ac.kr
SUPPLEMENTARY MATERIALS (3 Figures and 2 Tables)

Supplementary Figures

Supplementary Figure S1. Three different types of matches between a miRNA and its predicted mRNA targets categorized by TargetScan.

In addition to the core seed outlined with boxes, additional nucleotide can further be matched either at the position 8 (7mer-m8), the additional “A” preceding the seed sequence (7mer-A1), or both (8mer).

Supplementary Figure S2. Putative targeting of human WLS mRNA by hsa-miR-31-5p.

(A) The 3’-UTR regions of human WLS mRNA are probed for its putative match with the seed sequence of mature hsa-miR-31-5p. The cloning site for a luciferase assay is indicated with a blue
box. (B) The relative luciferase activity is compared among OSC20 cells transfected with 1) a construct containing WLS 3’UTR sequence (blue box in A) fused with luciferase domain (Luc-WLS target), 2) a WLS 3’UTR-containing construct as well as a miR-31 mimic (Luc-WLS target+miR-31), or 3) a WLS 3’UTR-containing construct, a miR-31 mimic and a synthetic single-stranded miR-31-inhibitor oligonucleotide complementary to mature miR-31 (Luc-WLS target+miR-31+miR-31-inhibitor). Mean± SEM indicated. **, P<0.01 and ***, P<0.001.

Supplementary Figure S3. Expression of Wnt pathway components in OSCC cells following transient transfection of miR-31.

(A and B) The protein levels of Wnt pathway components are shown in a western blot analysis 1) before and after transfection of miR-31 mimics in SAS and OSC20 cells (A) or 2) in a stable miR-31-expressing SAS and OSC20 cell lines (B).
Supplementary Tables

Table S1. Putative targets of dme-miR-31a/b predicted by TargetScanFly

| Target Gene ID | Gene symbol | Types of target sites |
|---------------|-------------|-----------------------|
|               |             | 8mer | 7mer-m8 | 7mer-A1 | Total |
| CG11247       | CG11247     | 1    | 0       | 1       | 2     |
| CG6210        | wls         | 0    | 1       | 0       | 1     |
| CG10079       | Egfr        | 1    | 0       | 0       | 1     |
| CG6634        | mld         | 1    | 0       | 0       | 1     |
| CG31240       | repo        | 1    | 0       | 0       | 1     |
| CG13209       | sha         | 1    | 0       | 0       | 1     |
| CG1136        | CG1136      | 1    | 0       | 0       | 1     |
| CG16947       | CG16947     | 1    | 0       | 0       | 1     |
| CG17390       | CG17390     | 0    | 0       | 1       | 1     |
| CG33106       | mask        | 0    | 0       | 2       | 2     |
| CG7852        | CG7852      | 0    | 0       | 1       | 1     |
| CG13287       | CG13287     | 0    | 0       | 1       | 1     |
| CG30429       | CG30429     | 0    | 1       | 0       | 1     |
| CG31243       | cpo         | 0    | 1       | 0       | 1     |
| CG32346       | E(bx)       | 0    | 1       | 0       | 1     |
| CG1864        | Hr38        | 0    | 1       | 0       | 1     |
| CG11770       | lin         | 0    | 1       | 0       | 1     |
| CG46186       | qkr54B      | 0    | 1       | 0       | 1     |
| CG4316        | Sb          | 0    | 1       | 0       | 1     |
| CG14223       | CG14223     | 0    | 1       | 0       | 1     |
| CG14767       | CG14767     | 0    | 1       | 0       | 1     |
| CG14837       | CG14837     | 0    | 1       | 0       | 1     |
| CG18641       | CG18641     | 0    | 1       | 0       | 1     |
| CG32446       | CG32446     | 0    | 1       | 0       | 1     |

Different sites of each gene putatively targeted by *Drosophila* miR-31 (dme-miR-31a/b) are listed.

The target sites determined by TargetScanFly (ver. 7.2) are categorized into three types: 7mer-A1, 7mer-m8 and 8mer sites (http://www.targetscan.org/docs/7mer.html; Supplementary Figure S1).
Table S2. Putative targets of hsa-miR-31 predicted by TargetScanHuman

| Target Gene Symbol | Gene name                                                                 | Types of target sites |
|--------------------|---------------------------------------------------------------------------|-----------------------|
| RNF144B            | ring finger protein 144B                                                  | 0 0 1 1               |
| RSN1               | round spermatid basic protein 1                                           | 1 1 0 2               |
| SH2D1A             | SH2 domain containing 1A                                                  | 1 0 0 1               |
| AK4                | adenylate kinase 4                                                        | 1 1 0 2               |
| PAX9               | paired box 9                                                              | 1 0 0 1               |
| LPP                | LIM domain containing preferred translocation partner in lipoma           | 0 0 2 2               |
| PRKCE              | protein kinase C, epsilon                                                 | 1 0 0 1               |
| NR5A2              | nuclear receptor subfamily 5, group A, member 2                           | 1 1 0 2               |
| TMEM145            | transmembrane protein 145                                                | 1 0 0 1               |
| ARHGEF2            | ho/Rac guanine nucleotide exchange factor (GEF) 2                         | 0 0 1 1               |
| WDR5               | WD repeat domain 5                                                        | 1 0 0 1               |
| PSMB11             | proteasome (prosome, macropain) subunit, beta type, 11                    | 1 0 0 1               |
| PEX5               | peroxisomal biogenesis factor 5                                           | 1 1 0 2               |
| FGF7               | fibroblast growth factor 7                                                | 1 0 0 1               |
| SYDE2              | synapase defective 1, Rho GTPase, homolog 2 (C. elegans)                 | 2 0 0 2               |
| PDZD2              | PDZ domain containing 2                                                   | 1 0 0 1               |
| SSH1               | slingshot protein phosphatase 1                                           | 1 0 0 1               |
| TFRC               | transferrin receptor                                                      | 1 0 0 1               |
| IL34               | interleukin 34                                                            | 1 0 0 1               |
| CIAPIN1            | cytokine induced apoptosis inhibitor 1                                     | 1 0 0 1               |
| PC                 | pyruvate carboxylase                                                      | 1 0 0 1               |
| CRYBG3             | beta-gamma crystallin domain containing 3                                 | 1 0 0 1               |
| EGLN3              | egl-9 family hypoxia-inducible factor 3                                    | 1 0 0 1               |
| TBX4A2R            | thromboxane A2 receptor                                                    | 1 0 0 1               |

Different sites of each gene putatively targeted by human miR-31 (hsa-miR-31) are listed. The target sites determined by TargetScanHuman (ver. 7.2) are categorized into three types: 7mer-A1, 7mer-m8 and 8mer sites (http://www.targetscan.org/docs/7mer.html; Supplementary Figure S1).