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

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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 \pm 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

Tangat Cana ID	Cana symbol	Types of target sites						
Target Gene ID	Gene symbol	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	сро	0	1	0	1			
CG32346	E(bx)	0	1	0	1			
CG1864	Hr38	0	1	0	1			
CG11770	lin	0	1	0	1			
CG4816	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			

Table	S1	Putative	targets	of dme	-miR_31a	/h ni	redicted	hv '	Targe	tScan	Flv
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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).

Target Gene		Types of target sites					
Symbol	Gene name	8mer	7mer-m8	7mer-A1	Total		
RNF144B	ring finger protein 144B	0	0	1	1		
RSBN1	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	synapse 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		
CIAPINI	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		
TBXA2R	thromboxane A2 receptor	1	0	0	1		

Table S2. Putative targets of hsa-miR-31 predicted by TargetScanHuman

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).