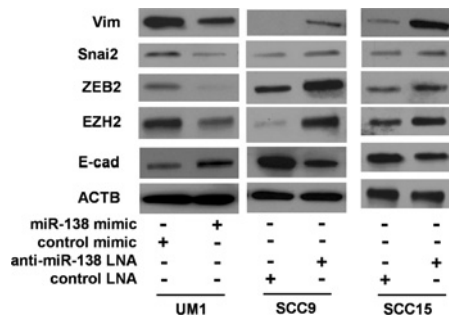


## SUPPLEMENTARY ONLINE DATA

# MicroRNA-138 suppresses epithelial–mesenchymal transition in squamous cell carcinoma cell lines

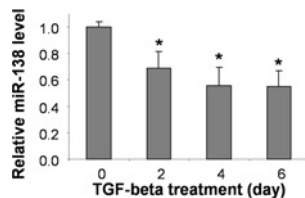
Xiqiang LIU\*<sup>†1</sup>, Cheng WANG\*<sup>†1</sup>, Zujian CHEN\*, Yi JIN\*, Yun WANG\*, Antonia KOLOKYTHAS\*<sup>‡§</sup>, Yang DAI<sup>§||</sup> and Xiaofeng ZHOU\*<sup>§¶2</sup>

\*Center for Molecular Biology of Oral Diseases, College of Dentistry, University of Illinois at Chicago, 801 South Paulina Street, Chicago, IL 60612, U.S.A., †Department of Oral and Maxillofacial Surgery, Guanghua School and Research Institute of Stomatology, Sun Yat-sen University, Guangzhou, Guangdong 510055, China, ‡Department of Oral and Maxillofacial Surgery, College of Dentistry, University of Illinois at Chicago, 801 South Paulina Street, Chicago, IL 60612, U.S.A., §Graduate College, University of Illinois at Chicago Cancer Center, University of Illinois at Chicago, 1801 West Taylor, Chicago, IL 60612, U.S.A., ||Department of Bioengineering, College of Engineering, University of Illinois at Chicago, 601 South Morgan Street, Chicago, IL 60607, U.S.A., and ¶Department of Periodontics, College of Dentistry, University of Illinois at Chicago, 801 South Paulina Street, Chicago, IL 60612, U.S.A.



**Figure S1** Effects of miR-138 on the expression of *VIM*, *SNAI2*, *ZEB2*, *EZH2* and *E-cad* genes in additional cell lines

UM1 Cells were transfected with miR-138 mimic or negative control mimic. The SCC9 and SCC15 cells were treated with anti-miR-138 LNA or negative control LNA. The expressional changes in *Vim*, *EZH2*, *ZEB2* and *E-cad* were measured in these cells by Western blot analysis.



**Figure S2** TGFβ-induced down-regulation of miR-138 expression

Cells were treated with vehicle or 10 ng/ml TGFβ for 2, 4 or 6 days. The changes in miR-138 was determined using the  $2^{-\Delta\Delta CT}$  method, where U6 was used as an internal reference. \*,  $P < 0.05$ . Error bars represent S.D.

<sup>1</sup> These authors contributed equally to this work.

<sup>2</sup> To whom correspondence should be addressed (email xfzhou@uic.edu).

**Table S1 EMT-related genes ( $n = 86$ ) that are differentially regulated by miR-138**

Total RNA was derived from three independent experiments. The fold changes (miR-138 mimic-treated compared with negative mimic-treated) of genes are calculated based on the EMT-specific qRT-PCR-based array analyses. A two-tailed Student's *t* test was used and the *P* value was represented. n/d, undetectable.

Gene symbol	GenBank® accession number	Description	Fold difference	<i>P</i> value	PicTar (4-way)	PicTar (5-way)	TargetScanS	TargetScan Human 5.1	miRanda (microrna.org)	miRanda (miRBase)	Number of positive predictions
<i>EZH2</i>	NM_004456	Enhancer of zeste homologue 2 ( <i>Drosophila</i> )	0.2434	0.001	1	1	1		1	1	5
<i>SERPINE1</i>	NM_000602	Serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1	0.3118	0.0092							0
<i>PLEK2</i>	NM_016445	Pleckstrin 2	0.3238	0.0653							0
<i>ZEB2</i>	NM_014795	Zinc finger E-box binding homeobox 2	0.3789	0.0222				1			1
<i>SNAI2</i>	NM_003068	Snail homologue 2 ( <i>Drosophila</i> )	0.4257	0.0012							0
<i>MMP3</i>	NM_002422	Matrix metalloproteinase 3 (stromelysin 1, progelatinase)	0.4258	0.0739							0
<i>RHOC</i>	NM_175744	Ras homologue gene family, member C	0.4353	0.0079	1		1	1	1	1	5
<i>VIM</i>	NM_003380	Vimentin	0.483	0.0045			1	1	1		3
<i>CDH2</i>	NM_001792	Cadherin 2, type 1, N-cadherin (neuronal)	0.4913	0.0775							0
<i>PTK2</i>	NM_005607	Protein tyrosine kinase 2	0.5113	0.0667	1	1	1	1			4
<i>STEAP1</i>	NM_012449	Six transmembrane epithelial antigen of the prostate 1	0.5216	0.0333							0
<i>TFPI2</i>	NM_006528	Tissue factor pathway inhibitor 2	0.5375	0.1144							0
<i>CAMK2n1</i>	NM_018584	Calcium/calmodulin-dependent protein kinase II inhibitor 1	0.5449	0.255							0
<i>EGFR</i>	NM_005228	Epidermal growth factor receptor [erythroblastic leukemia viral (v-erb-b) oncogene homologue, avian]	0.548	0.0639							0
<i>TGFB1</i>	NM_000660	Transforming growth factor $\beta$ 1	0.5694	0.0802							0
<i>IL1RN</i>	NM_000577	Interleukin 1 receptor antagonist	0.5732	0.2398							0
<i>OCLN</i>	NM_002538	Occludin	0.5819	0.0132							0
<i>TCF3</i>	NM_003200	Transcription factor 3 (E2A immunoglobulin enhancer binding factors E12/E47)	0.603	0.0007				1	1		2
<i>PPPDE2</i>	NM_015704	PPPDE peptidase domain containing 2	0.6063	0.0849							0
<i>SNAI1</i>	NM_005985	Snail homologue 1 ( <i>Drosophila</i> )	0.6199	0.0316							0
<i>KRT14</i>	NM_000526	Keratin 14	0.6472	0.2713							0
<i>NOTCH1</i>	NM_017617	Notch homologue 1, translocation-associated ( <i>Drosophila</i> )	0.648	0.1278							0
<i>TMEFF1</i>	NM_003692	Transmembrane protein with EGF-like and two follistatin-like domains 1	0.6556	0.1845							0
<i>MMP9</i>	NM_004994	Matrix metalloproteinase 9 (gelatinase B, 92 kDa gelatinase, 92 kDa type IV collagenase)	0.6883	0.1778							0
<i>GSK3B</i>	NM_002093	Glycogen synthase kinase 3 $\beta$	0.7449	0.2269							0
<i>CAV2</i>	NM_001233	Caveolin 2	0.745	0.0257							0
<i>NODAL</i>	NM_018055	Nodal homologue (mouse)	0.7858	0.4738							0
<i>SIP1</i>	NM_003616	Survival of motor neuron protein-interacting protein 1	0.8105	0.248							0
<i>CALD1</i>	NM_004342	Caldesmon 1	0.8137	0.4729							0
<i>ITGA5</i>	NM_002205	Integrin, $\alpha$ 5 (fibronectin receptor, $\alpha$ polypeptide)	0.8151	0.2809							0
<i>MAP1B</i>	NM_005909	Microtubule-associated protein 1B	0.8276	0.2825							0
<i>ITGB1</i>	NM_002211	Integrin $\beta$ 1 (fibronectin receptor, $\beta$ polypeptide, antigen CD29 includes MDF2, MSK12)	0.8303	0.1867							0

Table S1 Continued

Gen symbol	GenBank® accession number	Description	Fold difference	<i>P</i> value	PicTar (4-way)	PicTar (5-way)	TargetScanS	TargetScan Human 5.1	miRanda (microrna.org)	miRanda (miRBase)	Number of positive predictions
<i>AKT1</i>	NM_005163	V-akt murine thymoma viral oncogene homologue 1	0.8759	0.3845					1		1
<i>MMP2</i>	NM_004530	Matrix metalloproteinase 2 (gelatinase A, 72 kDa gelatinase, 72 kDa type IV collagenase)	0.882	0.5891							0
<i>ILK</i>	NM_004517	Integrin-linked kinase	0.8958	0.2931							0
<i>ZEB1</i>	NM_030751	Zinc finger E-box-binding homeobox 1	0.8974	0.1943							0
<i>TCF4</i>	NM_003199	Transcription factor 4	0.9216	0.5438				1			1
<i>MSN</i>	NM_002444	Moesin	0.9229	0.6739					1		1
<i>RAC1</i>	NM_006908	Ras-related C3 botulinum toxin substrate 1 (rho family, small GTP-binding protein Rac1)	0.9479	0.7117							0
<i>NUDT13</i>	NM_015901	Nudix (nucleoside diphosphate linked moiety X)-type motif 13	0.9791	0.899							0
<i>FN1</i>	NM_002026	Fibronectin 1	0.9877	0.8472							0
<i>PTP4A1</i>	NM_003463	Protein tyrosine phosphatase type IVA, member 1	0.9942	0.8591	1	1	1	1		1	5
<i>TMEM132A</i>	NM_178031	Transmembrane protein 132A	1.0133	0.8024					1		1
<i>DSP</i>	NM_004415	Desmoplakin	1.0176	1					1		1
<i>SOX10</i>	NM_006941	SRY (sex-determining region Y)-box 10	1.0267	0.5778							0
<i>BMP1</i>	NM_006129	Bone morphogenetic protein 1	1.051	0.9909					1		1
<i>MITF</i>	NM_000248	Microphthalmia-associated transcription factor	1.0835	0.3715							0
<i>SMAD2</i>	NM_005901	SMAD family member 2	1.0884	0.4472							0
<i>JAG1</i>	NM_000214	Jagged 1 (Alagille syndrome)	1.1339	0.963							0
<i>F11R</i>	NM_144503	F11 receptor	1.1934	0.3556							0
<i>CTNNB1</i>	NM_001904	Catenin (cadherin-associated protein), $\beta$ 1, 88 kDa	1.2017	0.1378							0
<i>ITGAV</i>	NM_002210	Integrin, $\alpha$ V (vitronectin receptor, $\alpha$ polypeptide, antigen CD51)	1.2314	0.0382							0
<i>TIMP1</i>	NM_003254	TIMP metalloproteinase inhibitor 1	1.26	0.1618							0
<i>AHNAK</i>	NM_024060	AHNAK nucleoprotein	1.3228	0.6767							0
<i>KRT7</i>	NM_005556	Keratin 7	1.3298	0.0331							0
<i>TGFB2</i>	NM_003238	Transforming growth factor $\beta$ 2	1.3303	0.0143							0
<i>MST1R</i>	NM_002447	Macrophage-stimulating 1 receptor (c-met-related tyrosine kinase)	1.4367	0.0786							0
<i>SPARC</i>	NM_003118	Secreted protein, acidic, cysteine-rich (osteonectin)	1.4568	0.0906							0
<i>KRT19</i>	NM_002276	Keratin 19	1.4854	0.0324							0
<i>VPS13A</i>	NM_033305	Vacuolar protein sorting 13 homologue A ( <i>Saccharomyces cerevisiae</i> )	1.5033	0.1345							0
<i>GSC</i>	NM_173849	Goosecoid homeobox	1.5039	0.8061							0
<i>WNT5A</i>	NM_003392	Wingless-type MMTV integration site family, member 5A	1.5716	0.0085							0
<i>DSC2</i>	NM_004949	Desmocollin 2	1.6013	0.128							0
<i>RG2</i>	NM_002923	Regulator of G-protein signalling 2, 24 kDa	1.6624	0.0403							0
<i>TGFB3</i>	NM_003239	Transforming growth factor $\beta$ 3	1.8351	0.0639							0
<i>BMP7</i>	NM_001719	Bone morphogenetic protein 7	1.908	0.1751							0
<i>FZD7</i>	NM_003507	Frizzled homologue 7 ( <i>Drosophila</i> )	1.96	0.1053							0
<i>STAT3</i>	NM_003150	Signal transducer and activator of transcription 3 (acute-phase response factor)	1.971	0.0585							0
<i>TWIST1</i>	NM_000474	Twist homologue 1 ( <i>Drosophila</i> )	2.0298	0.0087							0
<i>IGFBP4</i>	NM_001552	Insulin-like growth factor-binding protein 4	2.1158	0.0198							0

**Table S1 Continued**

Gen symbol	GenBank® accession number	Description	Fold difference	<i>P</i> value	PicTar (4-way)	PicTar (5-way)	TargetScanS	TargetScan Human 5.1	miRanda (microrna.org)	miRanda (miRBase)	Number of positive predictions
<i>TSPAN13</i>	NM_014399	Tetraspanin 13	2.1196	0.0174							0
<i>FOXC2</i>	NM_005251	Forkhead box C2 (MFH-1, mesenchyme forkhead 1)	2.1292	0.4907							0
<i>VCAN</i>	NM_004385	Versican	2.1428	0.0227							0
<i>COL1A2</i>	NM_000089	Collagen, type I, $\alpha 2$	2.2482	0.3691							0
<i>SNAI3</i>	NM_178310	Snail homologue 3 ( <i>Drosophila</i> )	2.3426	0.0726							0
<i>COL5A2</i>	NM_000393	Collagen, type V, $\alpha 2$	2.3567	0.008							0
<i>PDGFRB</i>	NM_002609	Platelet-derived growth factor receptor, $\beta$ polypeptide	2.3701	0.0755	1						1
<i>GNG11</i>	NM_004126	Guanine nucleotide binding protein (G protein), $\gamma 11$	2.4643	0.5945							0
<i>ERBB3</i>	NM_001982	V-erb-b2 erythroblastic leukemia viral oncogene homologue 3 (avian)	2.7155	0.0529							0
<i>SPP1</i>	NM_000582	Secreted phosphoprotein 1	3.6533	0.089							0
<i>FGFBP1</i>	NM_005130	Fibroblast growth factor binding protein 1	3.9607	0.0412							0
<i>WNT11</i>	NM_004626	Wingless-type MMTV integration site family, member 11	4.1513	0.059							0
<i>ESR1</i>	NM_000125	Estrogen receptor 1	4.2673	0.0146	1	1					2
<i>COL3A1</i>	NM_000090	Collagen, type III, $\alpha 1$	10.236	0.0689							0
<i>CDH1</i>	NM_004360	Cadherin 1, type 1, E-cadherin (epithelial)	12.958	0.0377							0
<i>WNT5B</i>	NM_032642	Wingless-type MMTV integration site family, member 5B	n/d	n/d							0

**Table S2 EMT-related genes that were down-regulated by miR-138 were significantly enriched with predicted candidate targets of miR-138**

The set of down-regulated transcripts was significantly enriched with candidate targets of miR-138 ( $\chi^2$  test,  $P$  value = 0.0278), whereas the set of up-regulated transcripts was not enriched with the candidate targets of miR-138 ( $P$  value = 1.0). A total of nine EMT-related genes were down-regulated ( $<0.5$ -fold difference and  $P < 0.10$ ), and 14 EMT-related genes were up-regulated ( $>2$ -fold difference and  $P < 0.10$ ) as measured by TaqMan-based qRT-PCR arrays which contain 86 EMT-related genes. The targets for miR-138 were predicted using our combined approach with six bioinformatics tools listed in the Material and methods section of the main text.

	Down-regulated by miR-138	Up-regulated by miR-138	Not regulated by miR-138
Number of genes	9	14	63
Number of predicted miR-138 target genes	4	2	9
Percentage of genes predicted as miR-138 targets	44.4%	14.3%	14.3%