Supplemental Information

Long Noncoding RNA RC3H2 Facilitates Cell

Proliferation and Invasion by Targeting

MicroRNA-101-3p/EZH2 Axis in OSCC

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Supplementary table 1. Clinical characteristic of the oral squamous cell carcinoma (OSCC) patients used for microarray assay.

NO.	Prior Radiotherapy	Age	Gender	Site	Clinical stage	TNM classification	Perineural invasion
1	No	56	Female	Gingiva	II	T2N0M0	No
2	No	54	Male	Gingiva	III	T3N0M0	No
3	No	58	Male	Tongue	III	T2N1M0	No
4	No	64	Male	Tongue	II	T2N0M0	Yes
5	No	57	Female	Tongue	IV	T4N0M0	No

TNM: tumor, node, metastasis

Supplementary table 2. Associations between RC3H2, EZH2 and miR-101-3p levels and clinical parameters in patients with OSCC (n = 34)

Group	RC3H2 expression (ΔCt Mean $\pm$ SD)	P value	EZH2 expression (ΔCt Mean $\pm$ SD)	P value	miR-101-3p expression (ΔCt Mean $\pm$ SD)	P value
Age(y)		0.3900		0.5799		0.9185
$\geq$ 60	0.1333 $\pm$ 0.02223		4.176 $\pm$ 1.506		3.472 $\pm$ 2.099	
< 60	0.1072 $\pm$ 0.0173		3.886 $\pm$ 0.9750		3.394 $\pm$ 1.743	
Gender		0.7831		0.6250		0.1812
Male	0.1253 $\pm$ 0.01934		4.189 $\pm$ 1.209		3.067 $\pm$ 1.959	
Female	0.1167 $\pm$ 0.02324		3.951 $\pm$ 1.595		3.995 $\pm$ 1.936	
Smoking history		0.3671		0.3496		0.7055
Yes	0.1385 $\pm$ 0.02915		3.770 $\pm$ 1.369		3.260 $\pm$ 1.684	
No	0.1096 $\pm$ 0.01704		4.245 $\pm$ 1.363		3.540 $\pm$ 2.130	
T stage		0.1280		0.8423		0.7201
T1-T2	0.1138 $\pm$ 0.01359		4.073 $\pm$ 1.416		3.404 $\pm$ 2.028	
T3-T4	0.1829 $\pm$ 0.072		4.221 $\pm$ 1.023		3.789 $\pm$ 1.724	
Lymph node status		0.8412		0.7809		0.3267
pN positive	0.1245 $\pm$ 0.02071		4.039 $\pm$ 1.551		3.184 $\pm$ 2.067	
pN negative	0.1182 $\pm$ 0.01917		4.175 $\pm$ 1.042		3.878 $\pm$ 1.812	
TNM stage		0.6554		0.9483		0.5182
I–II	0.1101 $\pm$ 0.01904		4.112 $\pm$ 1.062		3.751 $\pm$ 1.831	
III–IV	0.1245 $\pm$ 0.02071		4.079 $\pm$ 1.526		3.284 $\pm$ 2.072	
Perineural invasion		0.5749		0.2179		0.2142
Yes	0.1034 $\pm$ 0.01763		4.616 $\pm$ 0.8577		4.216 $\pm$ 1.385	
No	0.1241 $\pm$ 0.01853		3.929 $\pm$ 1.460		3.213 $\pm$ 2.090	
ECS		0.5775		0.9643		0.0524
Yes	0.1018 $\pm$ 0.03633		4.114 $\pm$ 0.9345		4.859 $\pm$ 1.003	
No	0.1236 $\pm$ 0.01662		4.086 $\pm$ 1.452		3.147 $\pm$ 2.011	
Prior Radiotherapy		0.6388		0.2893		0.2659
Yes	0.09724 $\pm$ 0.03292		4.899 $\pm$ 0.9517		2.221 $\pm$ 3.641	
No	0.1219 $\pm$ 0.01621		4.013 $\pm$ 1.383		3.568 $\pm$ 1.801	

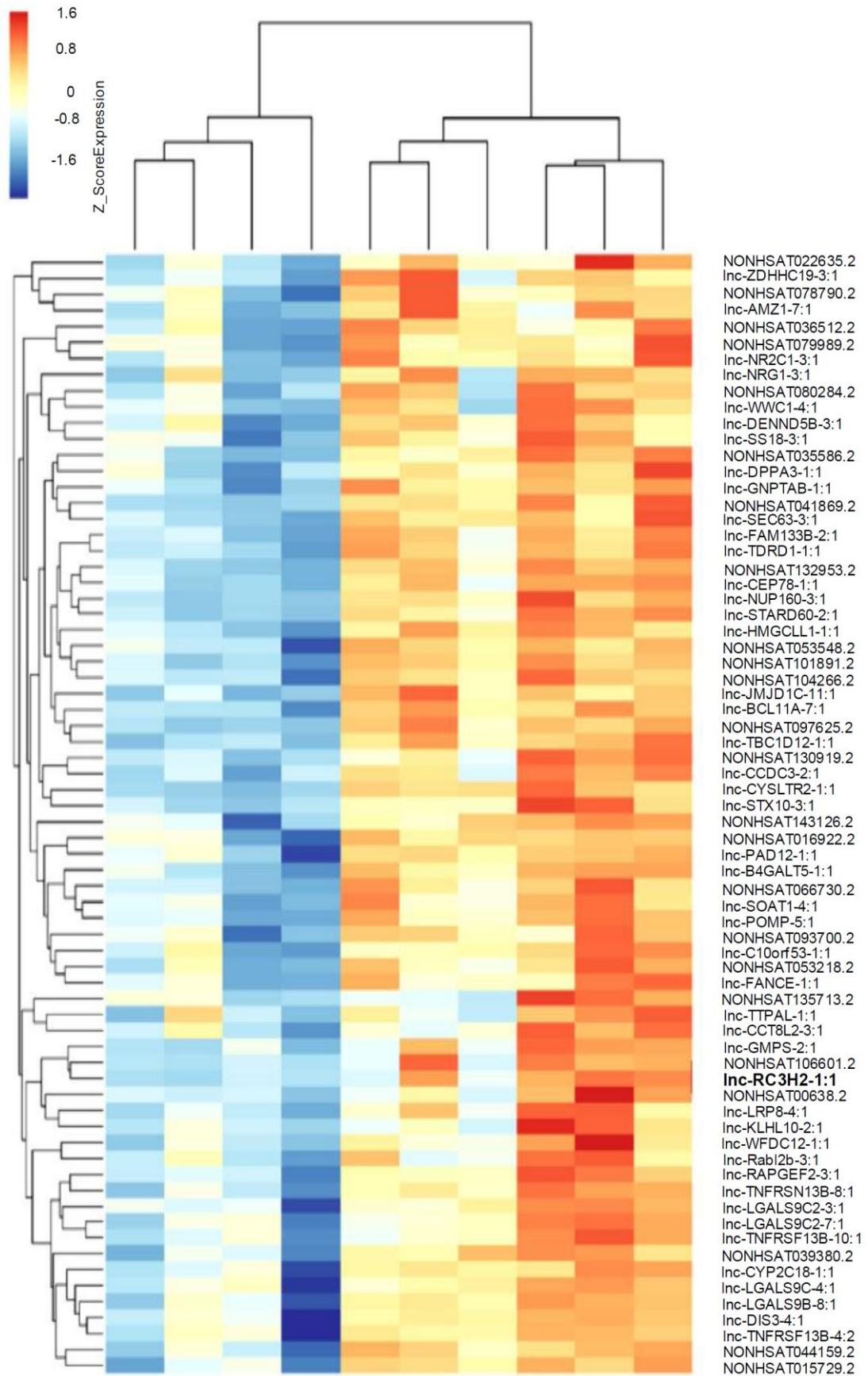Abbreviations: SD, standard deviation; pN, lymph node-metastasis stage. ECS, extracapsular spread of metastatic lymph nodes.

ΔCt indicates the difference in the cycle number at which a sample's fluorescent signal passes a given threshold above baseline (Ct) derived from a specific gene compared with that of GAPDH in tumor tissues.

Supplementary table 3. Sequence of qRT-PCR

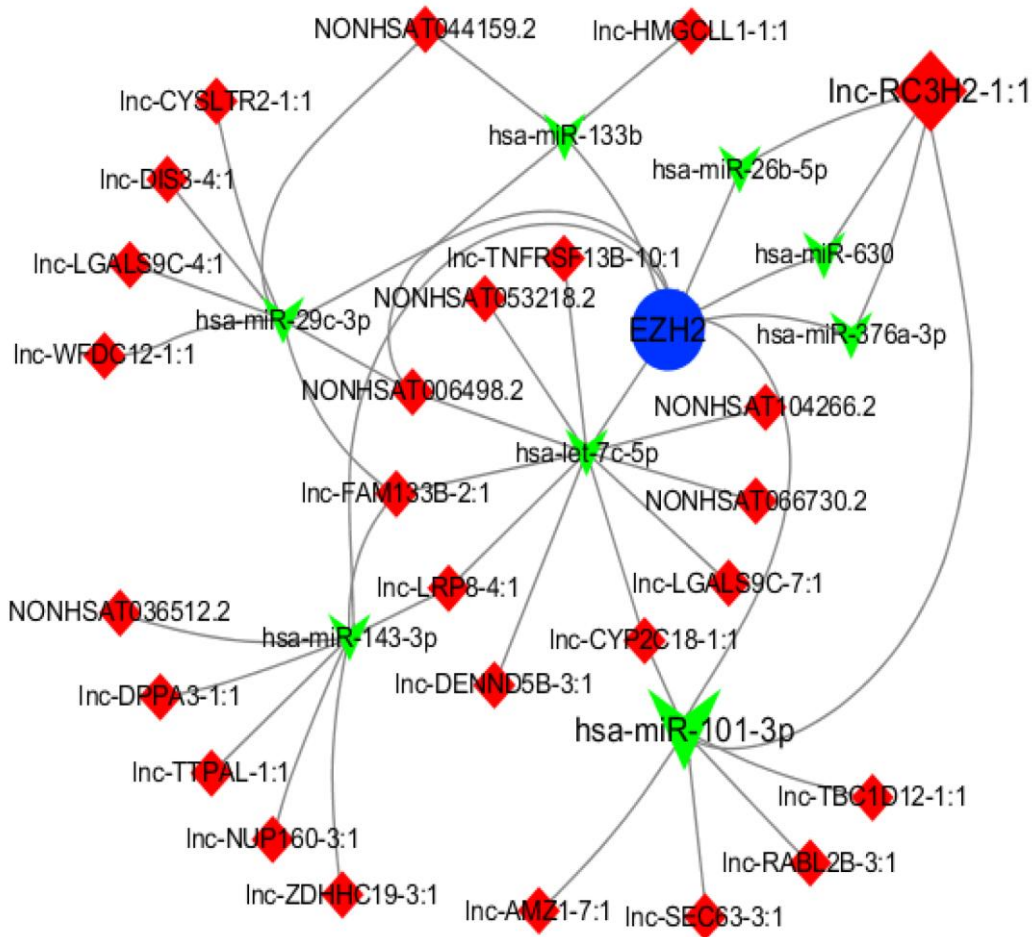| Primers	Forward	Reverse
RC3H2	CAACCGATTGTGAAGCTCTAGTG	CTTAGAGATGGTGCCTGCTTTTTA
GAPDH	GAACGGGAAGCTCACTGG	GAACGGGAAGCTCACTGG
EZH2	AATCAGAGTACATGCGACTGAGA	GCTGTATCCTTCGCTGTTTCC
U6	CTCGCTTCGGCAGCACATATACT	ATTTGCGTGTCATCCTTGCGCA
miR-101-3p	GCCGAGTACAGTACTGTGA	CTCAACTGGTGTCGTGGA

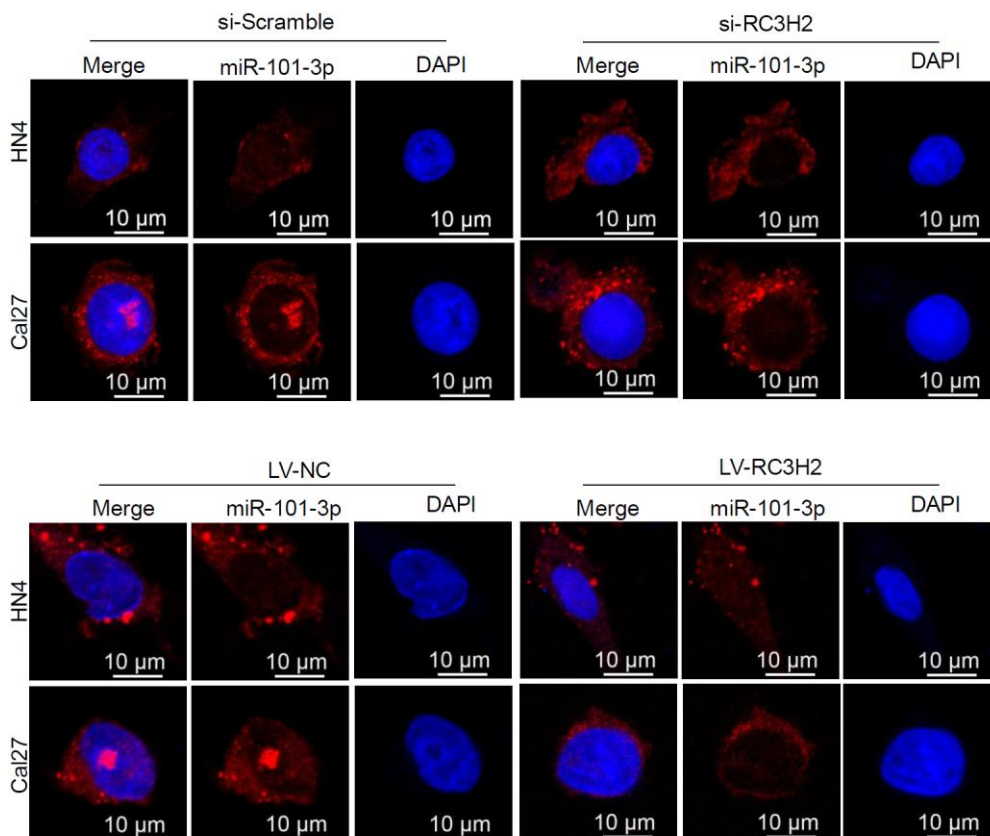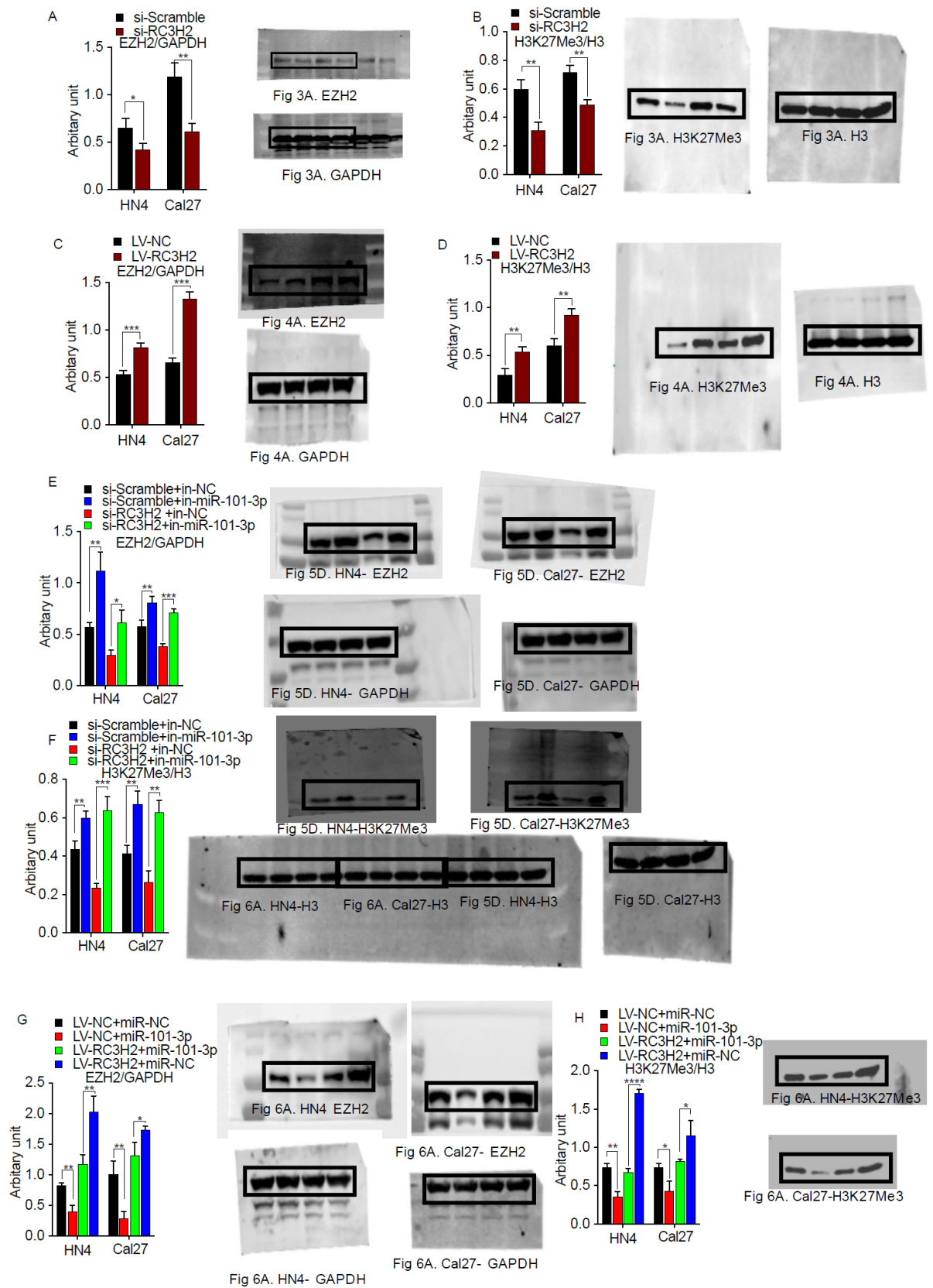## Supplementary Figure 1

Supplementary Figure 2

Supplementary Figure 3

Supplementary Figure 4

A

si-Scramble ■
si-RC3H2 ■
EZH2/GAPDH

Fig 3A. EZH2

Fig 3A. GAPDH

B

si-Scramble ■
si-RC3H2 ■
H3K27Me3/H3

Fig 3A. H3K27Me3   Fig 3A. H3

C

LV-NC ■
LV-RC3H2 ■
EZH2/GAPDH

Fig 4A. EZH2

Fig 4A. GAPDH

D

LV-NC ■
LV-RC3H2 ■
H3K27Me3/H3

Fig 4A. H3K27Me3   Fig 4A. H3

E

si-Scramble+in-NC ■
si-Scramble+in-miR-101-3p ■
si-RC3H2 +in-NC ■
si-RC3H2+in-miR-101-3p ■
EZH2/GAPDH

Fig 5D. HN4- EZH2   Fig 5D. Cal27- EZH2

Fig 5D. HN4- GAPDH   Fig 5D. Cal27- GAPDH

F

si-Scramble+in-NC ■
si-Scramble+in-miR-101-3p ■
si-RC3H2 +in-NC ■
si-RC3H2+in-miR-101-3p ■
H3K27Me3/H3

Fig 5D. HN4-H3K27Me3   Fig 5D. Cal27-H3K27Me3

Fig 6A. HN4-H3   Fig 6A. Cal27-H3   Fig 5D. HN4-H3   Fig 5D. Cal27-H3

G

LV-NC+miR-NC ■
LV-NC+miR-101-3p ■
LV-RC3H2+miR-101-3p ■
LV-RC3H2+miR-NC ■
EZH2/GAPDH

Fig 6A. HN4 EZH2

Fig 6A. Cal27- EZH2

Fig 6A. HN4- GAPDH

Fig 6A. Cal27- GAPDH

H

LV-NC+miR-NC ■
LV-NC+miR-101-3p ■
LV-RC3H2+miR-101-3p ■
LV-RC3H2+miR-NC ■
H3K27Me3/H3

Fig 6A. HN4-H3K27Me3

Fig 6A. Cal27-H3K27Me3

Supplementary Figure 5

Fig 3 I. CDKN2A

Fig 4 I. CDKN2A

Fig 3 I. MMP7

Fig 4 I. MMP7

Fig 3 I. TIMP2

Fig 4 I. TIMP2

Fig 3 I. MMP1

Fig 4 I. MMP1

Fig 3 I. CDH1

Fig 4 I. CDH1

Fig 3 I. GAPDH

Fig 4 I. GAPDH