

SUPPLEMENTARY INFORMATION

TITLE: DYSREGULATION OF miR-196b IN HEAD AND NECK CANCERS LEADS TO PLEIOTROPIC EFFECTS IN THE TUMOR CELLS AND SURROUNDING STROMAL FIBROBLASTS

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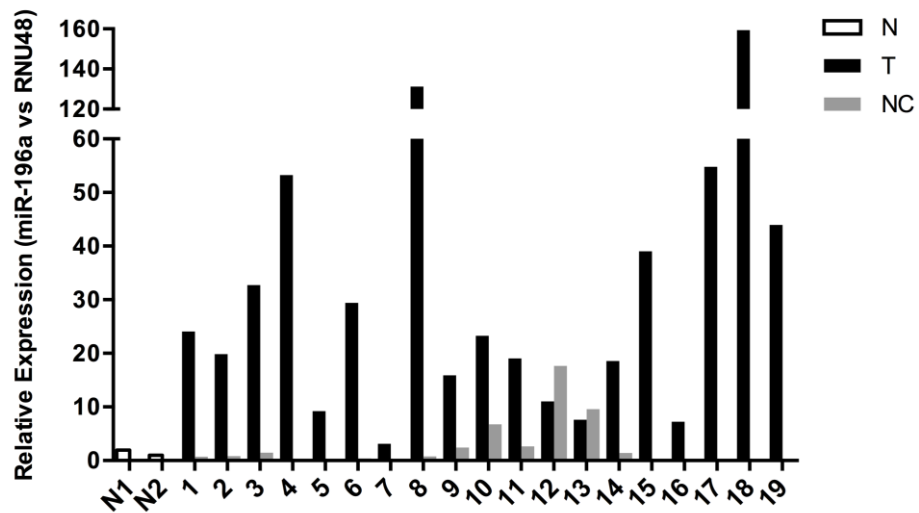
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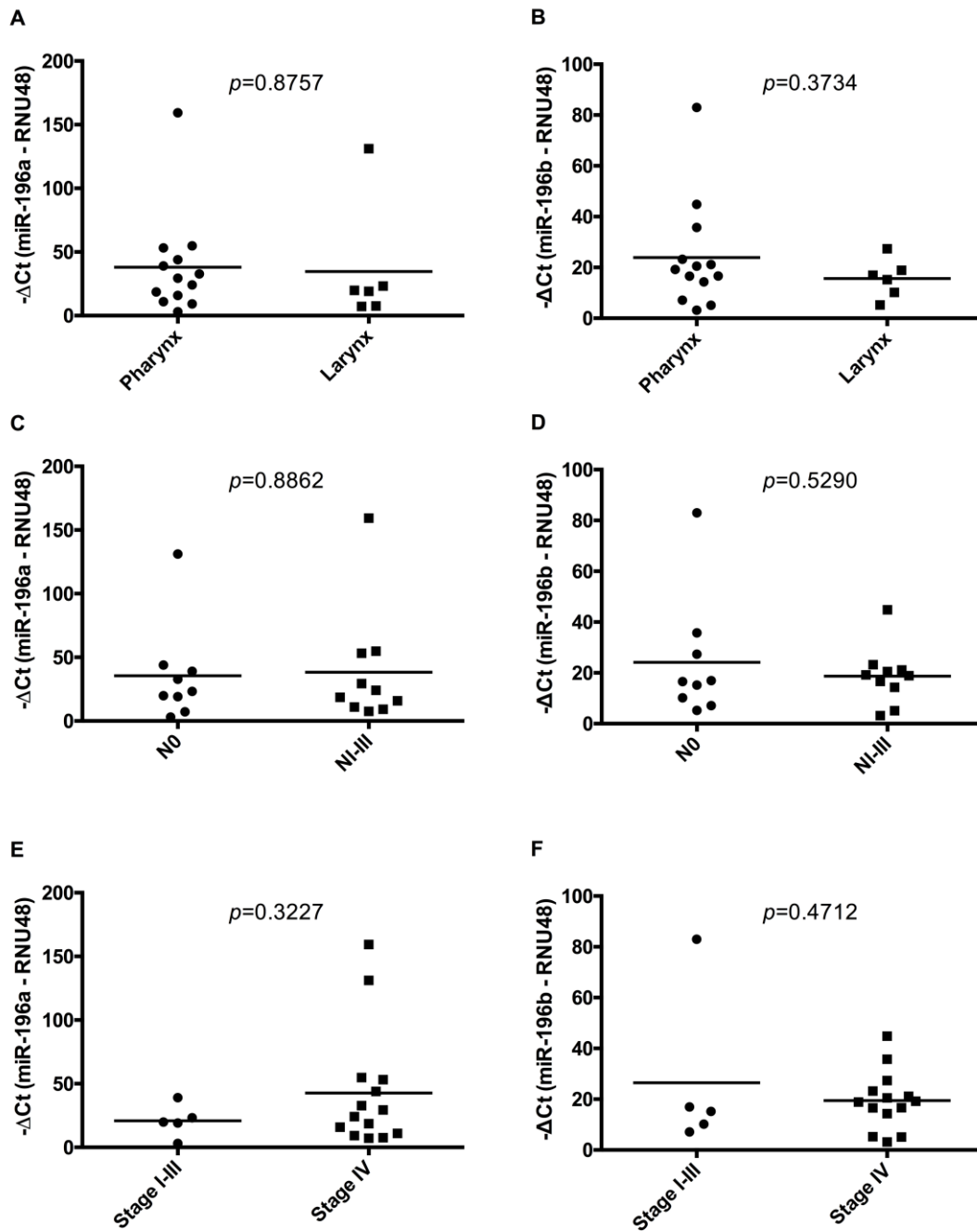
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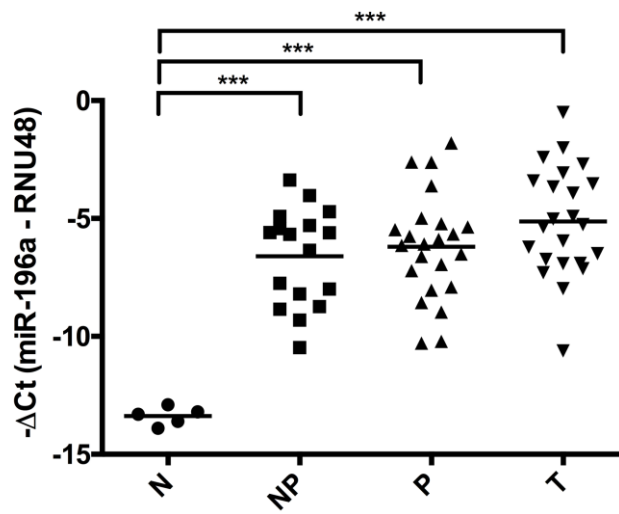
Supplementary Figure S1

Analysis of miR-196a expression in HNSCC tissue specimens. miR-196a expression levels were quantified by RT-qPCR in 19 fresh primary tumors (T) and patient-matched normal counterparts (NC). Data were normalized to RNU48 levels, and relative to the normal mucosa from non-oncologic patients (N).



Supplementary Figure S2

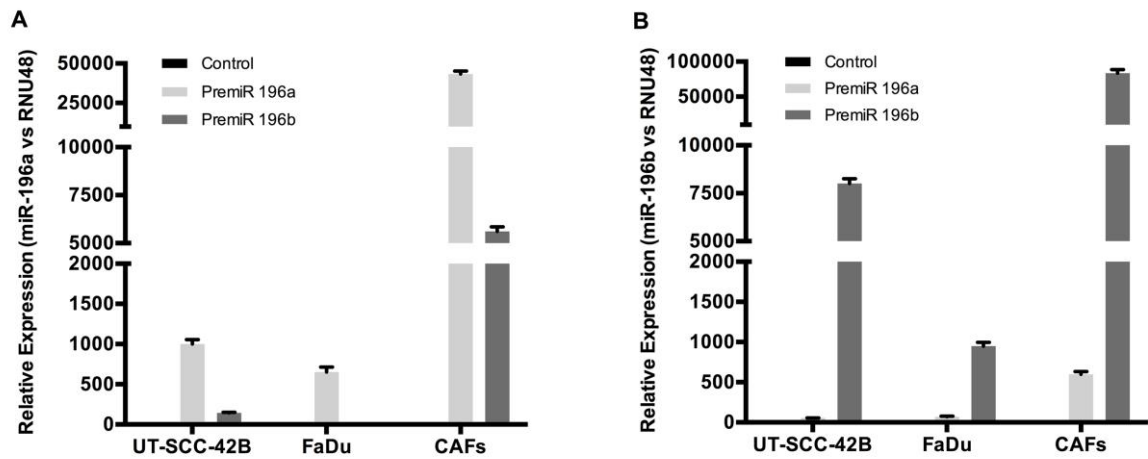
miR-196a and miR-196b levels in HNSCC tissue specimens distributed according to the tumor site (**A and B**), pN classification (**C and D**), and disease stage (**E and F**). *p* values calculated by Student t-test.



Supplementary Figure S3

Analysis of miR-196a expression in early stages of HNSCC tumorigenesis. miR-196a expression levels were quantified by RT-qPCR in 40 patients with laryngeal precancerous lesions, comprising 17 non-progressing dysplasias (NP), 23 progressing dysplasias (P) and the 23 patient-matched invasive tumors (T) subsequently developed. Normal epithelia from 5 non-oncologic patients were included as (N) healthy controls.

*** $p < 0.001$ by Holm-Sidak's multiple comparisons test.



Supplementary Figure S4

Analysis of miR-196a and miR-196b expression in HNSCC cells and CAFs transfected with premiR precursors. **(A)** miR-196a and **(B)** miR-196b expression levels were quantified by qRT-PCR in UT-SCC-42B, FaDu or CAFs transfected with either premiR-196a, premiR-196b or non-targeting control. Data were normalized to RNU48 levels and relative to control-transfected cells. The graphs represent the mean \pm SD of at least three independent experiments run in triplicate.

Supplementary Table S1. Clinicopathologic characteristics of the HNSCC patients
(N=19)

Characteristic	No. (%)
- Mean age, years	60
- Gender	
Male	17 (89)
Female	2 (11)
- pT classification	
T1-T2	5 (26)
T3	5 (26)
T4	9 (47)
- pN classification	
N0	9 (47)
N1-3	10 (53)
- Disease stage	
I-II	3
III	2
IV	14
- Pathological grade	
Well differentiated	9 (47)
Moderately differentiated	3 (16)
Poorly differentiated	7 (37)
- Site	
Pharynx	13 (68)
Larynx	6 (32)
Total Cases	19

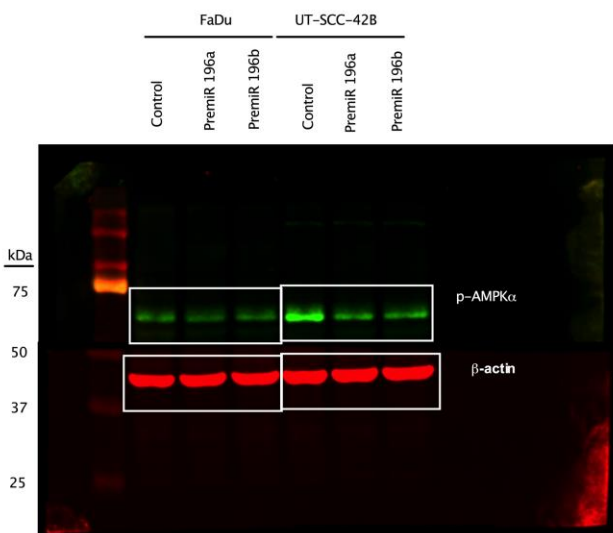
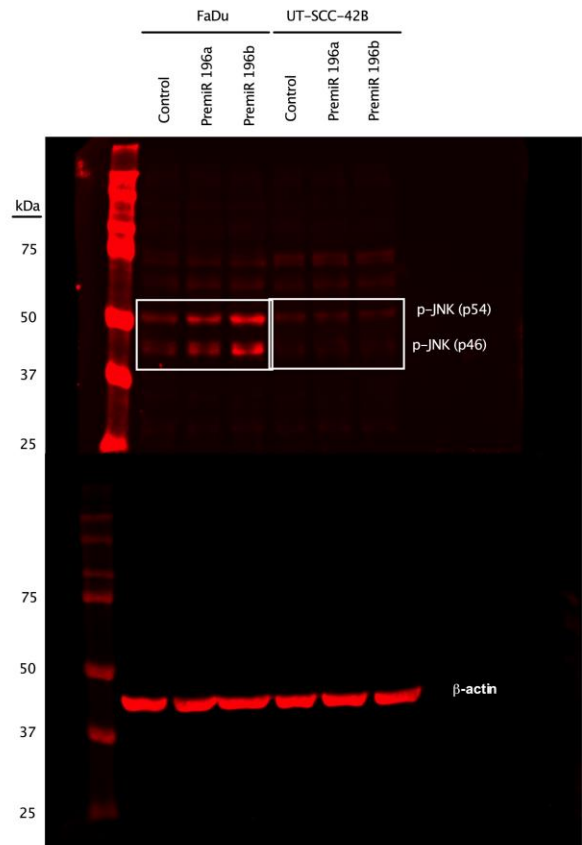
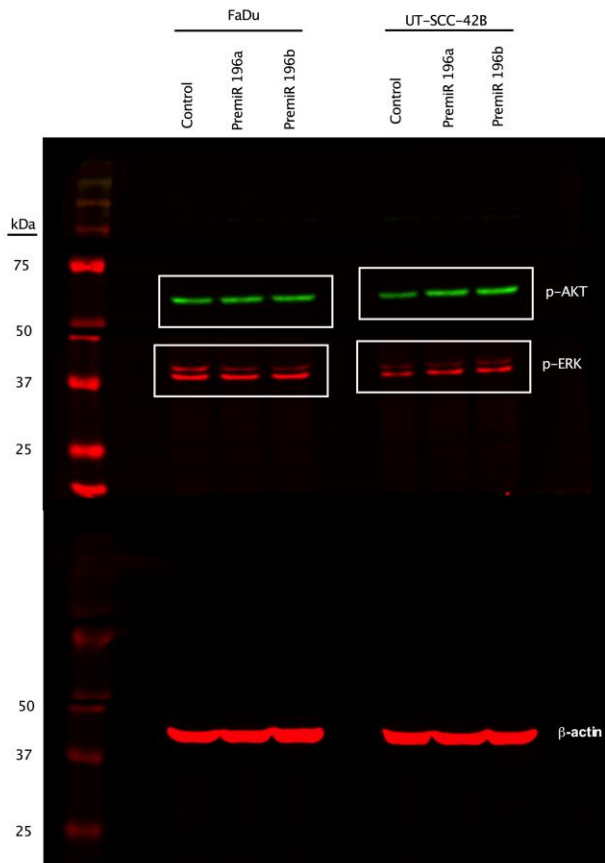
Supplementary Table S2. Clinicopathologic characteristics of the premalignant patients (N=40)

Characteristic	No. (%)
- Mean age, years	65
- Gender	
Male	36 (90)
Female	4 (10)
- Grade classification	
Low-grade	17 (42)
High-grade	23 (58)
Total Cases	40

Supplementary Table S3

Primers used for real-time RT-PCR (5' → 3')

ANXA1-Fw	GCAGGCCTGGTTTATTGAAA
ANXA1-Rv	GCTGTGCATTGTTTCGCTTA
BACH1-Fw	CTGATGGAGAGCTGAACATTACTCTT
BACH1-Rv	AGCAGTGTAGGCAAACCTGAATTAAG
FASLG-Fw	AGAGTCTACCAGCCAGAT
FASLG-Rv	AGTTGGACTTGCCTGTTA
FAS-Fw	ATGGCCAATTCTGCCATAAG
FAS-Rv	GACAAAGCCACCCCAAGTTA
HMGA1-Fw	ACAGCGCTGGTAGGGAGTCA
HMGA1-Rv	CCCCGAGGTCTCTTAGGTGTT
HMOX1-Fw	TGACCCGAGACGGCTTCA
HMOX1-Rv	CCTCCAGGGCCACATAGATG
HOXA7-Fw	TACCCCTGGATGCGGTCTT
HOXA7-Rv	CAGGTAGCGGTTGAAGTGGAA
HOXC8-Fw	TCCAGCCTCATGTTTCCAT
HOXC8-Rv	TCTGATACCGGCTGTAAGTTTGC
HOXD8-Fw	CTCGTCTCCTTCTCAAATGTTTCC
HOXD8-Rv	GACTGTAGGTTTGTCTTCCTCTTCGT
KRT5-Fw	CCGCAGCCGGACAGAA
KRT5-Rv	TGTCTGCTGCAGCTCCTCAT
NFKB1-Fw	TGCCAACAGATGGCCCATAC
NFKB1-Rv	TGTTCTTTTCACTAGAGGCACCA
RPL19-Fw	GCGGAAGGGTACAGCCAAT
RPL19-Rv	GCAGCCGGCGCAAA
S100A9-Fw	TGTGGCTCCTCGGCTTTG
S100A9-Rv	GCGTTCAGCTGCGACAT
TNF-Fw	CCAGGCAGTCAGATCATCTTCTC
TNF-Rv	AGCTGGTTATCTCTCAGCTCCAC



Uncropped membranes for Figure 6b