

Title Page

Title: Overexpression of miR-499-5p inhibits non-small cell lung cancer proliferation and metastasis by targeting VAV3

Authors' names: Ming Li¹, Shenjun Zhang², Ning Wu³, Liang Wu⁴, Changhui Wang¹, Yinying Lin^{1,2*}

Authors' affiliations:

¹Department of Respiratory Medicine, Shanghai 10th People's Hospital, Tongji University, Shanghai 200072, China.

²Institute of Biochemistry and Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, China.

³Department of Thoracic Surgery, Huashan Hospital, Fudan University, Shanghai 200032, China.

⁴Department of Thoracic Surgery, Shanghai Pulmonary Hospital, Tongji University, Shanghai 200433, China.

Corresponding author: Y.Lin. Shanghai 10th People's Hospital, Tongji University, Shanghai 200072, China; Institute of Biochemistry and Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, China.

Email: linyinping69@163.com

Fax number: 86-021-66306430, Telephone: 86-021-66306430

Keywords: Lung cancer; miR-499-5p; VAV3; Metastasis.

Figure S1

miR-499-5p expression levels in NSCLC cell lines.

(A) qRT-PCR data show lower expression of miR-499-5p in NSCLC cell lines (A549, H23, H522 and HOP62) compared with human bronchial epithelial cells (BEAS-2B). Data are presented as mean \pm s.d. (B) Correlation scatter plot of miR-499-5p and VAV3 expression in A549 and H23 cells.

Figure S2

(A) Quantitation of BrdU staining results. Error bars represent the mean \pm s.d. of three independent experiments. ***, $p < 0.001$. (B) Immunofluorescence analysis of BrdU incorporation in A549 cells transfected with pre-miR-499-5p vs control vs ASO-miR-499 for 72 h.

Figure S3

miR-499-5p induces cell cycle arrest.

In miR-499-5p or NC stably transfected-A549 and H23 cells, cells in different cycle phases are detected. Cell cycle was examined by FACS. A significant increase in S-phase was observed. ** $P < 0.01$.

Figure S4

A549 and H23 cells were transfected with pre-miR-499, miR-499-5p mimic and miR-499-3p, respectively. pre-miR-499 and miR-499-5p mimic inhibited NSCLC cells proliferation. Error bars represent the mean \pm s.d. of three independent experiments. **, $p < 0.01$; ***, $p < 0.001$.

Figure S5

Expression of ECM and EMT-related markers in NSCLC cells.

(A) Protein expression level of MMP-2, MMP-9 and β -actin in A549 H23 cells transfected with vector, miR-499-5p and miR-499-5p inhibitor was detected by western blotting analysis. (B) Western blot analysis shows VAV3 and miR-499-5p regulates E-cadherin and vimentin expression in A549 cells.

Figure S6

The efficiency of overexpression and knockdown of miR-499-5p.

qRT-PCR was performed to confirmed the miR-499-5p expression in A549 cells after transfection with pre-miR-499 and ASO-miR-499. Error bars represent the mean \pm s.d. of three independent experiments. ***, $p < 0.001$.

Figure S7

Sequence alignment of miR-499-5p with the 3' UTR of the VAV3 gene.

Figure s1

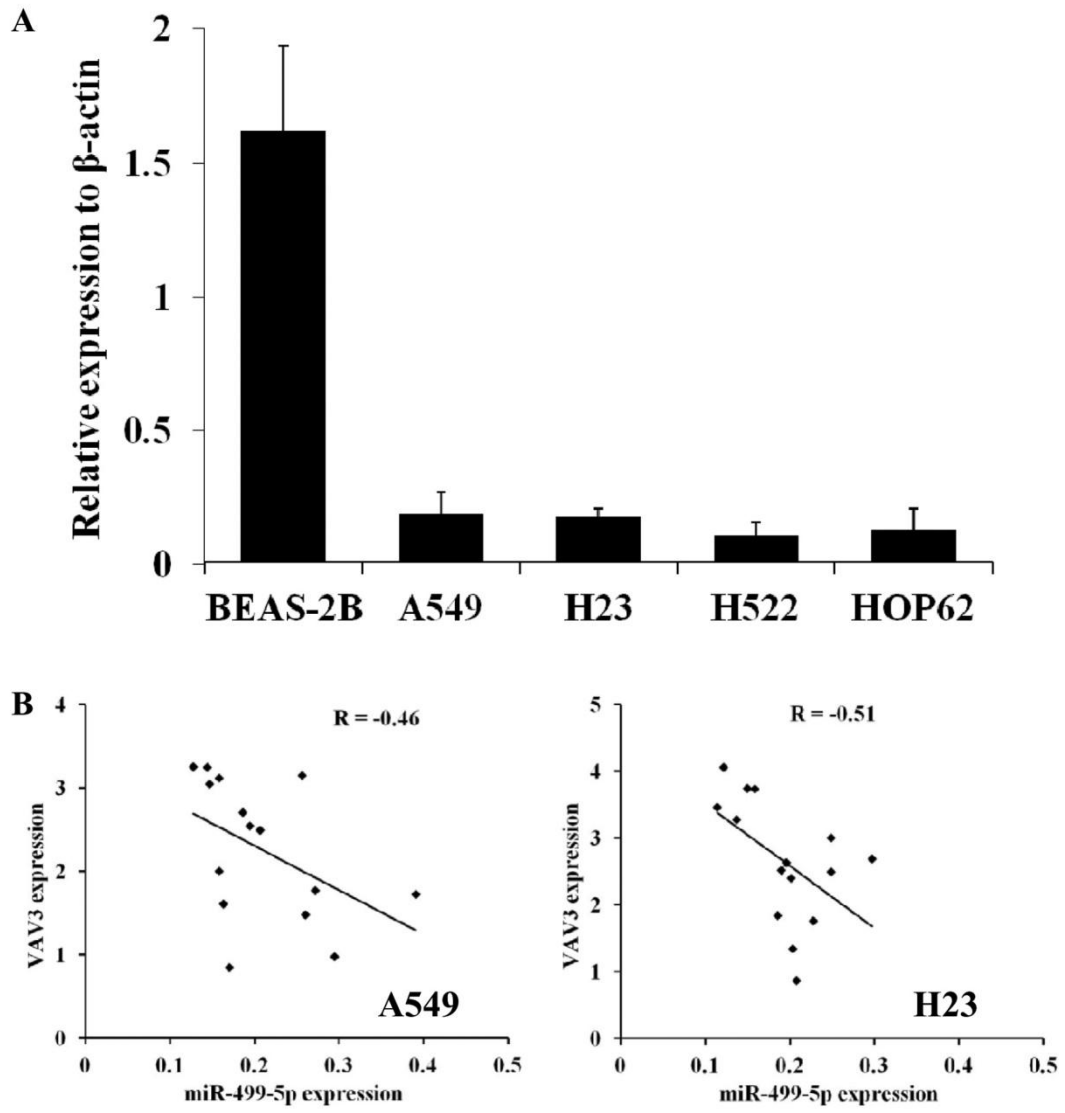


Figure s2

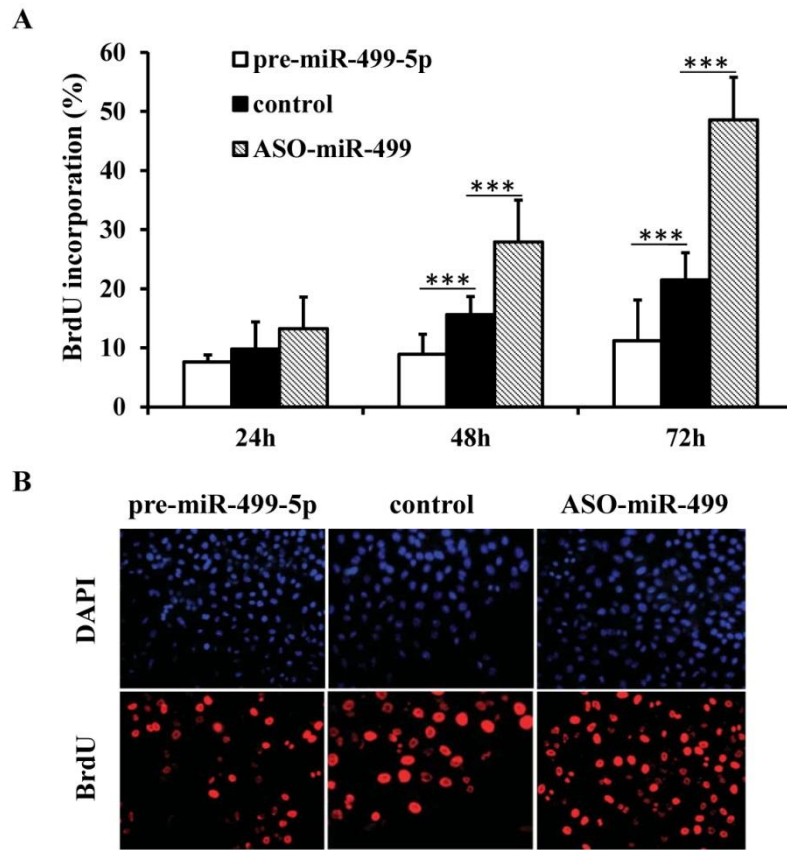


Figure s3

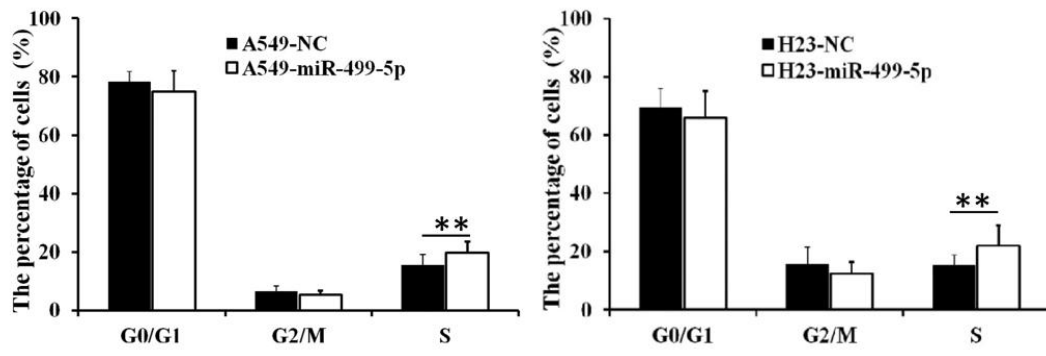


Figure s4

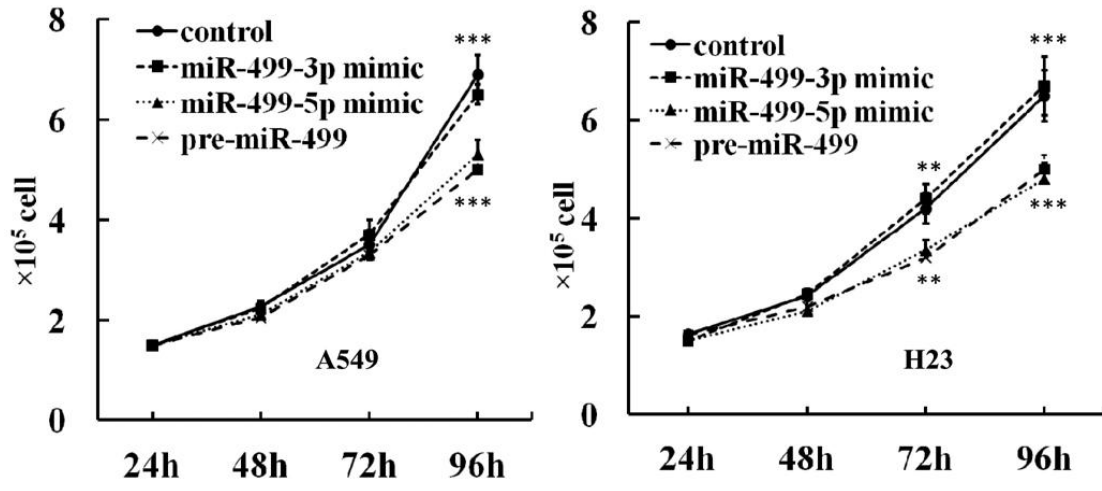


Figure s5

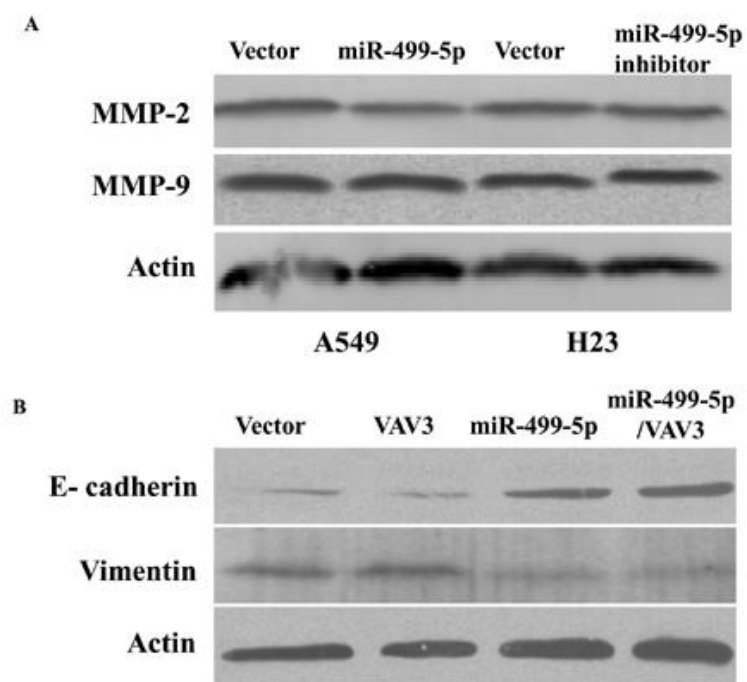


Figure s6

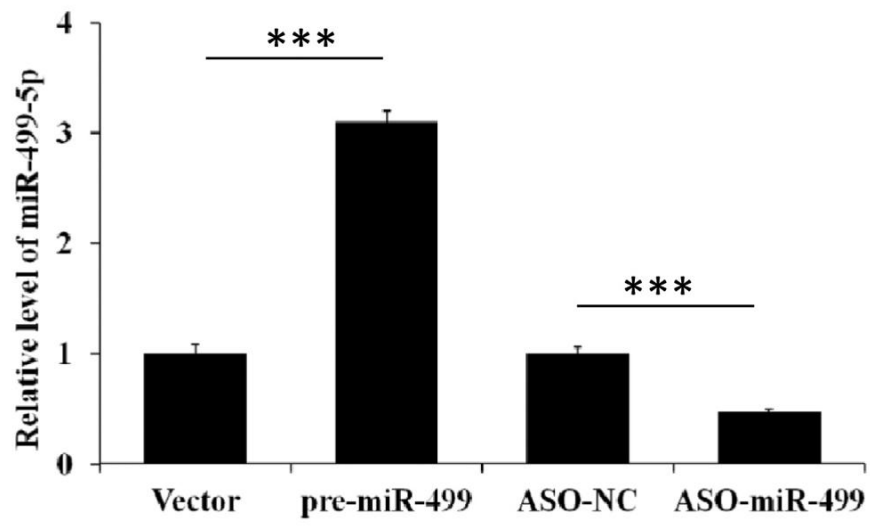


Figure s7

