**The long noncoding RNA LOC105374325 causes podocyte injury in individuals with focal segmental glomerulosclerosis**

Shuai Hu[1#], Runhong Han[1,2#], Jingsong Shi[1], Xiaodong Zhu[1], Weisong Qin[1], Caihong Zeng[1], Hao Bao[1*] and Zhihong Liu[1*]

[1] *National Clinical Research Center of Kidney Diseases, Jinling Hospital, Nanjing University School of Medicine, Nanjing, 210002, China*
[2] *School of Medicine, Southeast University, Nanjing, 210009, China*

**Running title:** *Role of lncRNA LOC105374325 in podocytes of FSGS*

**Correspondence:**
[*] Hao Bao, M.D., National Clinical Research Center of Kidney Diseases, Jinling Hospital, Nanjing University School of Medicine, 305 East Zhong Shan Road, Nanjing 210002, China. Phone: 86-25-84801992; Fax: 86-25-84801992; E-mail: bhao@nju.edu.cn
[*] Zhihong Liu, M.D., National Clinical Research Center of Kidney Diseases, Jinling Hospital, Nanjing University School of Medicine, 305 East Zhong Shan Road, Nanjing 210002, China. Phone: 86-25-84801992; Fax: 86-25-84801992; E-mail: zhihong--liu@hotmail.com

[#] Co-first authors
[*] Co-corresponding authors

**Supporting information**
**Table S1.** Oligomers used in this study
**Table S2.** Antibodies used in this study
**Figure S1.** Absolute quantification of LOC105374325, miR-34c and miR-196a/b in podocytes.
**Figure S2.** The binding between mmu-miR-34c and mmu-Bax 3' UTR and the binding between mmu-miR-196a/b and mmu-Bak 3' UTR.

**Tables**

**Table S1. Oligomers used in this study**

| Name | Application | Primer list |
|---|---|---|
| LOC105374325-F | PCR | GTCACCTGTGGCCCTATTT |
| LOC105374325-R | PCR | CAGACGAGGCCATAGAAGTTAG |
| hsa-ACTB-F | PCR | GACCTGACTGACTACCTCATGAAGAT |
| hsa-ACTB-R | PCR | GTCACACTTCATGATGGAGTTGAAGG |
| hsa-Bax-F | PCR | CCCGAGAGGTCTTTTTCCGAG |
| hsa-Bax-R | PCR | CCAGCCCATGATGGTTCTGAT |
| hsa-Bak-F | PCR | CATCAACCGACGCTATGACTC |
| hsa-Bak-R | PCR | GTCAGGCCATGCTGGTAGAC |
| hsa-pri-miR-34c-F | PCR | TGGGGTACCAACTTGAGACTG |
| hsa-pri-miR-34c-R | PCR | TTTGGACGAATTCCCAAATC |
| hsa-pri-miR-196a-F | PCR | AGTAACTGCCGTGAATCGGG |
| hsa-pri-miR-196a-R | PCR | GTTAAGCCGCTTTTTGGCCC |
| hsa-pri-miR-196b-F | PCR | GCTGTCGAGAGAAAGGTGGA |
| hsa-pri-miR-196b-R | PCR | CCCCTTCCTTGACGCATTTG |
| mmu-pri-miR-34c-F | PCR | AGTCTGAGAGCACCAGCTAAA |
| mmu-pri-miR-34c-R | PCR | CTATGGCTCTGTCCTCACCA |
| mmu-pri-miR-196a-F | PCR | CCGGGACTGTTGAGTGAAGT |
| mmu-pri-miR-196a-R | PCR | TTTTGCAGGGGGAACTGACA |
| mmu-pri-miR-196b-F | PCR | GCTGTCGAGAGAAAGGTGGA |
| mmu-pri-miR-196b-R | PCR | AGGGTGGTGTCTGGTACAGG |
| mmu-ACTB-F | PCR | GGCTGTATTCCCCTCCATCG |
| mmu-ACTB-R | PCR | CCAGTTGGTAACAATGCCATGT |
| LOC105374325-DNA-sense | pull down | (biotin-)GGTCCAGGGGACAGCTTGCCAGGA |
| LOC105374325-DNA-antisense | pull down | (biotin-)TCCTGGCAAGCTGTCCCCTGGACC |
| ChIP-C/EBPβ-F | ChIP | CAGCAAAGGAACGGAACAGA |
| ChIP-C/EBPβ-R | ChIP | TGATGGATGTTCATGGCTCAC |

**Table S2. Antibodies used in this study**

| Antibody | Catalog no. | Company | Reactivity |
|---|---|---|---|
| BAX | ab32503 | Abcam | Human, Mouse |
| BAK | 12105 | CST | Human, Mouse |
| Phospho-p38 | 4511 | CST | Human |
| p38 | ab31828 | Abcam | Human |
| p-C/EBPβ | ab52194 | Abcam | Human |
| C/EBPβ | PA5-27244 | Invitrogen | Human |
| β-tubulin | ab6046 | Abcam | Human |
| WT1 | ab89901 | Abcam | Human, Mouse |
| β-actin | ab8227 | Abcam | Human |

## Figures

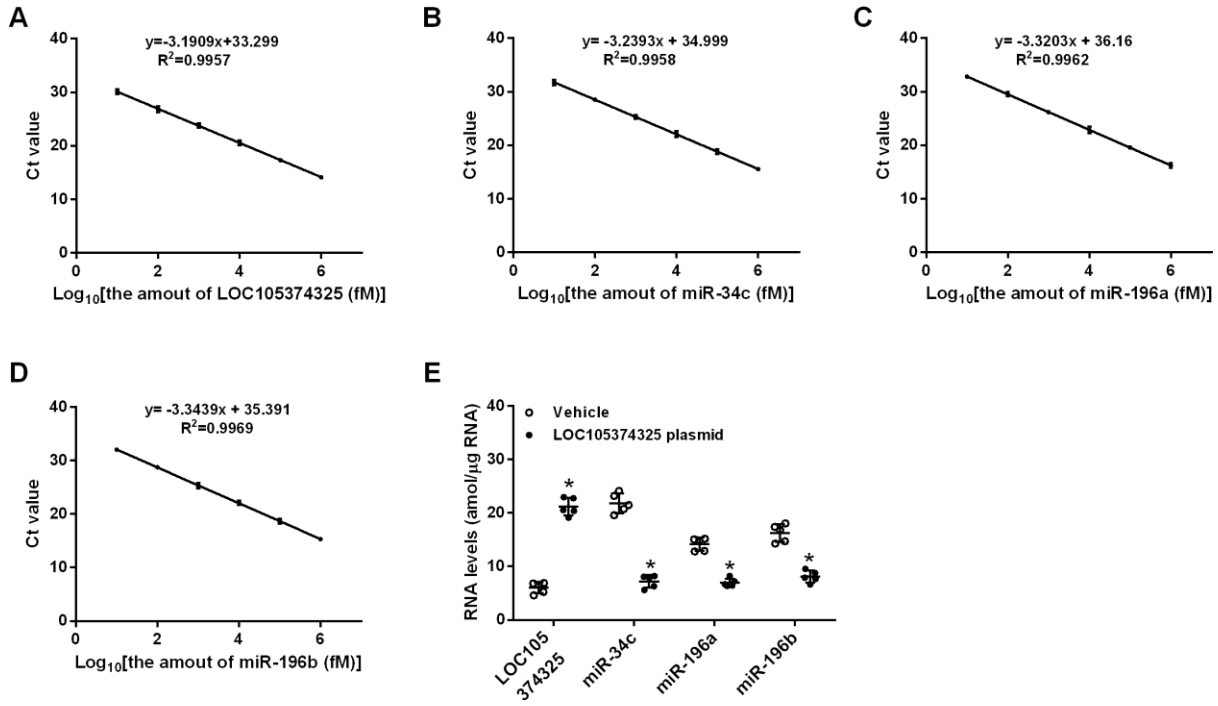


**Figure S1. Absolute quantification of LOC105374325, miR-34c and miR-196a/b in podocytes (A-D),** The standard curves of LOC105374325, miR-34c and miR-196a/b generated by linear regression analysis of $\log_{10}$(concentration) and Ct values; **(E)**, Change of absolute level of LOC105374325, miR-34c and miR-196a/b in podocytes transfected LOC105374325 plasmid (n=5). For statistical analysis, a two-tailed Student's t-test was used for **E**. *, $P<0.05$ compared with control.

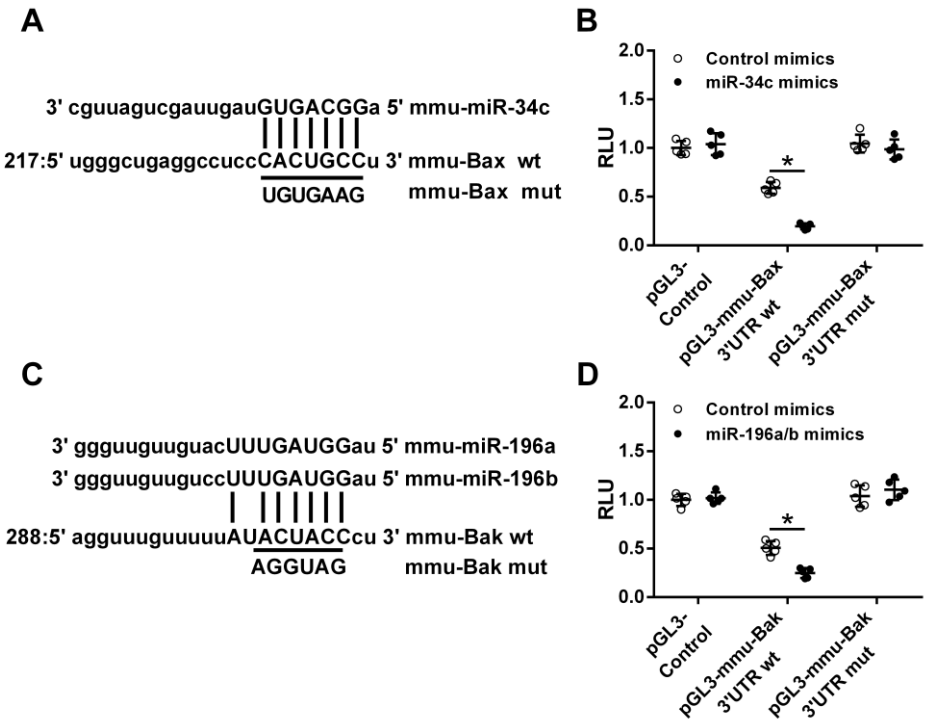


**Figure S2. The binding between mmu-miR-34c and mmu-Bax 3' UTR and the binding between mmu-miR-196a/b and mmu-Bak 3' UTR** **(A)** The binding site in the 3' UTR of mmu-Bax mRNA targeted by miR-34c; **(B)** Normalized luciferase activity of reporter constructs containing the 3' UTR of mmu-Bax or mutant 3' UTR of mmu-Bax in podocytes cotransfected with miR-34c mimics (n=5); **(C)** The binding site in the 3' UTR of mmu-Bak mRNA targeted by miR-196a/b; **(D)** Normalized luciferase activity of reporter constructs containing the 3' UTR of mmu-Bak or mutant 3' UTR of mmu-Bak in podocytes cotransfected with miR-196a/b mimics (n=5). For statistical analysis, a two-tailed Student's t-test was used for **B** and **D**. *, $P<0.05$ compared with cells transfected with pGL3-mmu-Bax 3'UTR wt or pGL3-mmu-Bak 3'UTR wt plasmid.