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N6-methyladenosine-modified circNfix attenuates sepsis-induced acute kidney injury by facilitating the ubiquitination of YTHDF2 and increasing DAPK2 mRNA stability

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Objectives : Increasing evidence shows that circRNAs play an important role in sepsis-induced acute kidney injury (SAKI). However, the mechanisms of circRNAs in SAKI have not been reported except the way of miRNA sponges. Notably, the function of N^6-methyladenosine (m^6A)-modified circRNA in SAKI remains undiscovered. Therefore, global profiles of circRNAs in kidneys with SAKI are needed, which will contribute to uncovering novel mechanisms underlying circRNAs in pathophysiology of SAKI.
Methods : SAKI-associated circRNAs were screened via circRNA microarray and examined using in situ hybridization (ISH) in renal tissue. The role of circNfix in YTHDF2-induced pro-inflammatory or pro-apoptotic response of tubular epithelial cells (TECs) was assesed by luciferase reporter assay and Annexin V/ PI staining assay in vitro. Adeno-associated virus was used to deliver circNfix to the kidneys of SAKI mice to study the function of circNfix in vivo. The mechanism underlying circNfix-mediated activation of NF-κB signaling was examined by Mass spectrometry, RNA pull-down, MeRIP-qPCR and ISH.
Results : circNfix expression was downregulated in TECs following SAKI, leading to apoptosis and inflammatory injury in TECs in vitro and in vivo. The downregulation of circNfix expression was post-transcriptionally attributed to the RNA splicing regulator RBM47. Mechanistically, circNfix was found to provide a scaffold for the interaction between YTHDF2 and HECTD1, which was augmented by circNfix N^6-methyladenosine (m^6A) modification, facilitating YTHDF2 ubiquitination and degradation. By decreasing the expression of YTHDF2, circNfix promoted the stabilization of DAPK2 mRNA, a negative regulator of NF-κB signaling, to protect LPS-induced TECs injury. Finally, restoring circNfix in an experimentally-induced mouse SAKI model suppressed NF-κB signaling, thereby improving renal function and ameliorating tubular injury.
Conclusions : Collectively, m^6A modified-circNfix suppresses NF-κB-driven renal inflammation via a YTHDF2-dependent mechanism during SAKI, and restoring circNfix might represent a therapeutic strategy for SAKI.

Figure 1.jpg

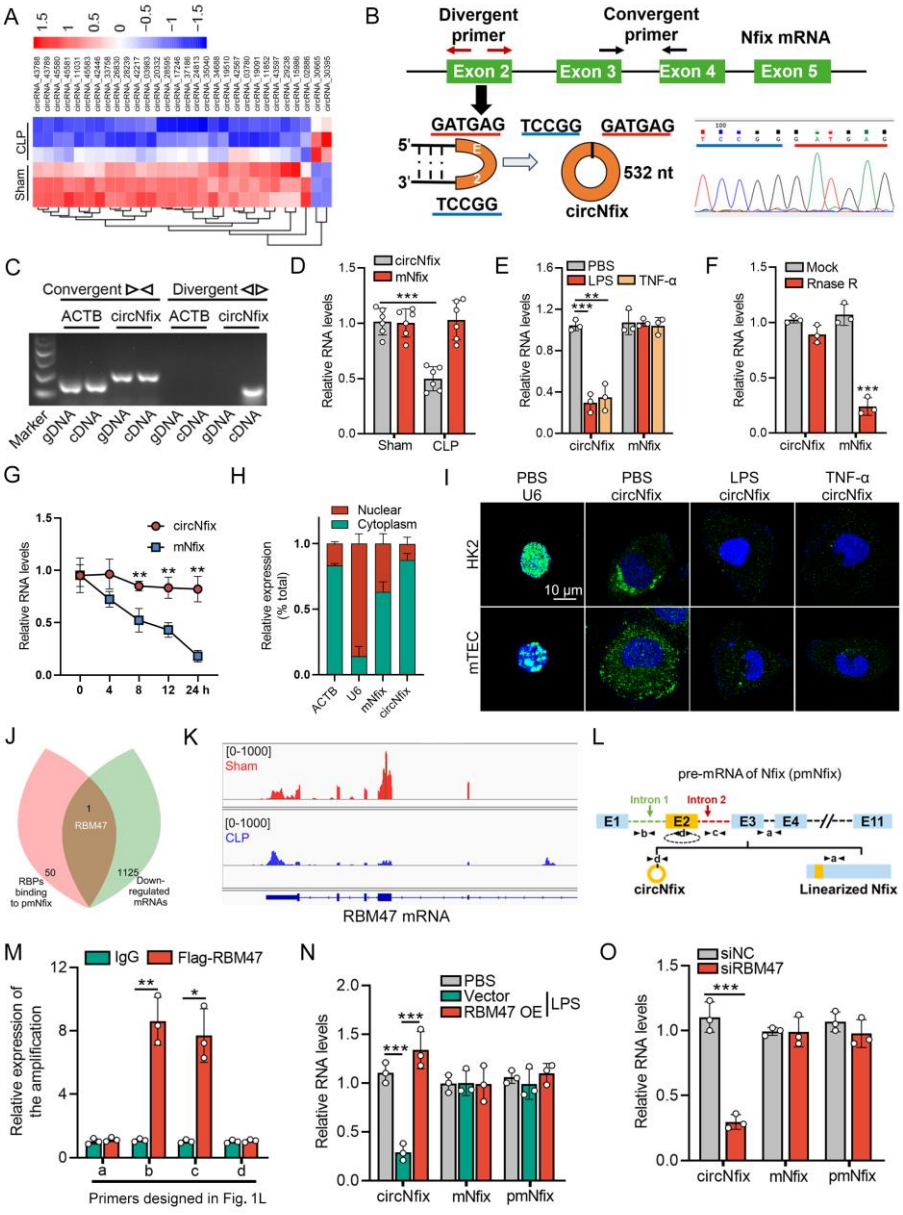
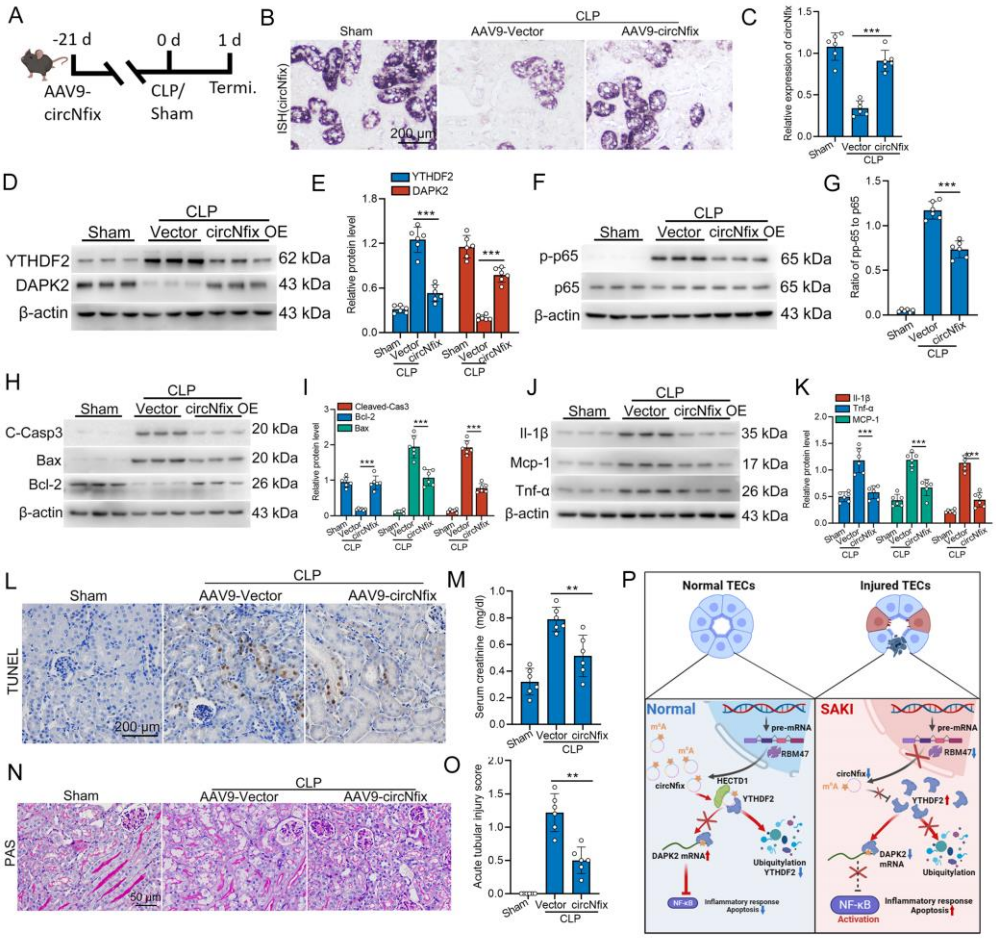

A
CLP
Sham
B
Divergent primer
Convergent primer
Nfix mRNA
Exon 2
Exon 3
Exon 4
Exon 5
GATGAG
TCCGG
GATGAG
5'
3'
TCCGG
532 nt
circNfix
C
Convergent
Divergent
ACTB
circNfix
ACTB
circNfix
Marker
gDNA
cDNA
gDNA
cDNA
gDNA
cDNA
gDNA
cDNA
D
circNfix
mNfix
Relative RNA levels
Sham
CLP
E
PBS
LPS
TNF-α
Relative RNA levels
circNfix
mNfix
F
Mock
Rnase R
Relative RNA levels
circNfix
mNfix
G
circNfix
mNfix
Relative RNA levels
0
4
8
12
24 h
H
Nuclear
Cytoplasm
Relative expression (% total)
ACTB
U6
mNfix
circNfix
I
PBS U6
PBS circNfix
LPS circNfix
TNF-α circNfix
HK2
mTEC
10 μm
J
RBM47
1
50
1125
RBPs binding to pmNfix
Down-regulated mRNAs
K
[0-1000]
Sham
[0-1000]
CLP
RBM47 mRNA
L
pre-mRNA of Nfix (pmNfix)
Intron 1
Intron 2
E1
E2
E3
E4
E11
circNfix
Linearized Nfix
M
IgG
Flag-RBM47
Relative expression of the amplification
a
b
c
d
Primers designed in Fig. 1L
N
PBS
Vector
RBM47 OE
LPS
Relative RNA levels
circNfix
mNfix
pmNfix
O
siNC
siRBM47
Relative RNA levels
circNfix
mNfix
pmNfix

Figure 1.jpg


A
-21 d
0 d
1 d
AAV9-circNfix
CLP/ Sham
Termi.
B
Sham
CLP
AAV9-Vector
AAV9-circNfix
ISH(circNfix)
200 μm
C
Relative expression of circNfix
Sham
Vector
circNfix
CLP
D
Sham
CLP
Vector
circNfix OE
YTHDF2
DAPK2
β-actin
62 kDa
43 kDa
43 kDa
E
YTHDF2
DAPK2
Relative protein level
F
p-p65
p65
β-actin
65 kDa
65 kDa
43 kDa
G
Ratio of pp-65 to p65
H
C-Casp3
Bax
Bcl-2
β-actin
20 kDa
20 kDa
26 kDa
43 kDa
I
Cleaved-Cas3
Bcl-2
Bax
J
Il-1β
Mcp-1
Tnf-α
β-actin
35 kDa
17 kDa
26 kDa
43 kDa
K
Il-1β
Tnf-α
MCP-1
L
TUNEL
200 μm
M
Serum creatinine (mg/dl)
N
PAS
50 μm
O
Acute tubular injury score
P
Normal TECs
Injured TECs
Normal
SAKI
pre-mRNA
RBM47
circNfix
HECTD1
YTHDF2
DAPK2 mRNA
Ubiquitylation YTHDF2
NF-κB
Inflammatory response
Apoptosis
DAPK2 mRNA
Ubiquitylation
NF-κB Activation