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**Supplementary Material for**

# New therapeutic method for alleviating damage of acute kidney injury through *BCL-2* gene promoter i-motif

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**Table S1.** Primer sequence information

| Spics | Genes | Forward primer (5′-3′) | Reverse primer (5′-3′) |
|---|---|---|---|
| human | BCL-2 | TGTTGTTCAAACGGGATTCA | GGCTGGGCACATTTACTGTT |
| | BAX | AGCGACTGATGTCCCTGTCT | CTCAGCCCATCTTCTTCCAG |
| | β-actin | CTGGAACGGTGAAGGTGAA | AAGGGACTTCTGTAACAACGA |
| | siRNA 1 | GCAAAGUGCAACGUAUUGUUATT | UAACAAUACGUUGCACUUUGCTT |
| | siRNA 2 | CCUUCUUCGUUUAGACAUGAUTT | AUCAUGUCUAAACGAAGAAGGTT |
| | siRNA 3 | GCACUGAAUCACUAUCAGAUATT | UAUCUGAUAGUGAUUCAGUGCTT |
| mice | BCL-2 | GTACCTGAACCGGCATCTG | GGGGCCATATAGTTCCACAA |
| | BAX | TAGCAAACTGGTGCTCAAGG | TCTTGGATCCAGACAAGCAG |
| | β-actin | GACCTCTATGCCAACACAGTGC | GTACTCCTGCTTGCTGATCCAC |

**Table S2.** The qPCR raw data from real-time RT-PCR experiment for cells and animal samples

| Experiment 1 | Ct | | [a]-ΔΔCt [a] | [b]F = $2^{-\Delta\Delta Ct}$ |
|---|---|---|---|---|
| | β-actin | BCL-2 | | |
| Control | 13.26 | 25.28 | 0 | 1 |
| 3 mg/ml FA | 13.45 | 27.84 | 0.49 | 0.71 |
| 3 mg/ml FA +20 μM **A22** | 13.86 | 25.02 | 0.013 | 0.99 |
| 3 mg/ml FA + 40 μM **A22** | 12.82 | 25.26 | -0.43 | 1.35 |

| Experiment 2 | Ct | | [a]-ΔΔCt [a] | [b]F = $2^{-\Delta\Delta Ct}$ |
|---|---|---|---|---|
| | β-actin | hnRNP LL | | |
| Control | 25.14 | 18.69 | 0 | 1 |
| siRNA1 | 26.40 | 21.52 | 1.39 | 0.34 |
| siRNA2 | 28.31 | 22.51 | 0.47 | 0.63 |
| siRNA3 | 26.12 | 20.31 | 0.45 | 0.64 |
| | β-actin | BCL-2 | [a]-ΔΔCt [a] | [b]F = $2^{-\Delta\Delta Ct}$ |
| Control | 25.13 | 21.38 | 0 | 1 |
| siRNA1 | 26.39 | 24.27 | 1.07 | 1.0 |
| siRNA2 | 28.31 | 24.76 | -0.35 | 0.81 |
| siRNA3 | 25.45 | 21.26 | -0.49 | 0.89 |
| | β-actin | BAX | [a]-ΔΔCt [a] | [b]F = $2^{-\Delta\Delta Ct}$ |
| Control | 25.23 | 20.8 | 0 | 1 |
| siRNA1 | 26.40 | 21.69 | 0.05 | 1.21 |
| siRNA2 | 28.31 | 23.6 | 0.05 | 1.21 |
| siRNA3 | 26.12 | 21.58 | 0.33 | 0.93 |

| Experiment 3 | Ct | | [a]-ΔΔCt [a] | [b]F = $2^{-\Delta\Delta Ct}$ |
|---|---|---|---|---|
| | β-actin | BCL-2 | | |
| Control | 15.45 | 31.635 | 0 | 1 |
| FA 24h | 15.43 | 32.33 | 0.63 | 0.64 |
| FA 24h + 20 mg/kg **A22** | 15.97 | 31.35 | 0.62 | 0.65 |
| FA 24h + 40 mg/kg **A22** | 16.49 | 30.26 | 0.32 | 0.80 |
| FA 48h | 15.73 | 28.535 | 1.04 | 0.49 |
| FA 48h + 20 mg/kg **A22** | 15.88 | 31.35 | 0.65 | 0.63 |

| | | | | |
|---|---|---|---|---|
| FA 48h + 40 mg/kg **A22** | 15.73 | 30.26 | 0.4 | 0.75 |
| | β-actin | BAX | [a]-ΔΔCt [a] | [b]F = $2^{-\Delta\Delta Ct}$ |
| Control | 16.16 | 20.45 | 0 | 1 |
| FA 24h | 15.52 | 18.91 | -0.68 | 1.60 |
| FA 24h + 20 mg/kg **A22** | 15.99 | 19.88 | -0.26 | 1.20 |
| FA 24h + 40 mg/kg **A22** | 15.53 | 19.54 | -0.103 | 1.07 |
| FA 48h | 16.60 | 19.56 | -1.17 | 2.25 |
| FA 48h + 20 mg/kg **A22** | 15.60 | 19.3 | -0.42 | 1.34 |
| FA 48h + 40 mg/kg **A22** | 15.79 | 19.82 | -0.1 | 1.07 |

[a] **-ΔΔCt** = [(Ct value of genes in treated sample - Ct value of genes in control) - (Ct value of GADPH in treated sample - Ct value of GADPH in control)]. [b] F: The relative expression fold.
Experiment 1: The Ct values of *BCL-2* in 3 mg/ml FA model with different concentration of **A22**
Experiment 2: HK-2 cells were treated with different fragments of siRNA. The Ct values of hnRNP LL, BCL-2 and BAX were obtained.
Experiment 3: The Ct values of *BCL-2* and Bax in FA-induced and **A22** administration group of mice kidney.

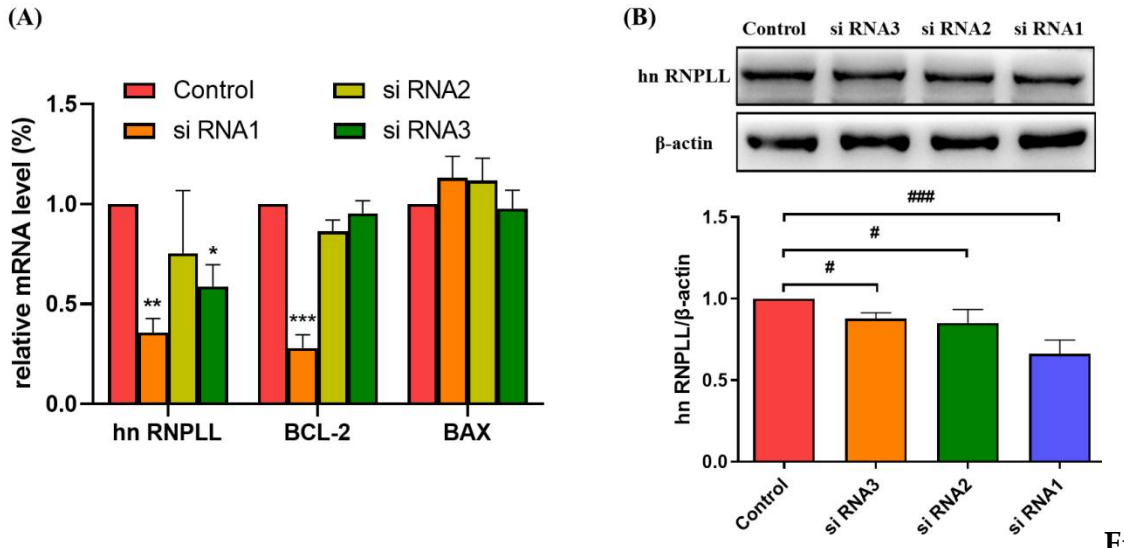


**Figure S1**. Screening of siRNA fragments for the best silencing effect and subsequent Western blot experiment. (A) The mRNA levels of hnRNP LL, BCL-2 and BAX. (*p<0.05, **p<0.01 and ***p<0.001 vs. Control group). (B) The changes of hnRNP LL in HK-2 cells and corresponding histogram after siRNA silencing with oligomers of different sequences. The extracted proteins from the cells were immunoblotted with specific antibodies, and quantified based on the loading control of ACTIN ($^{###}$p<0.001, vs. Control group). These data showed that siRNA1 had a better silencing effect compared to other siRNA fragments, so the fragment of siRNA1 was selected for target verification.

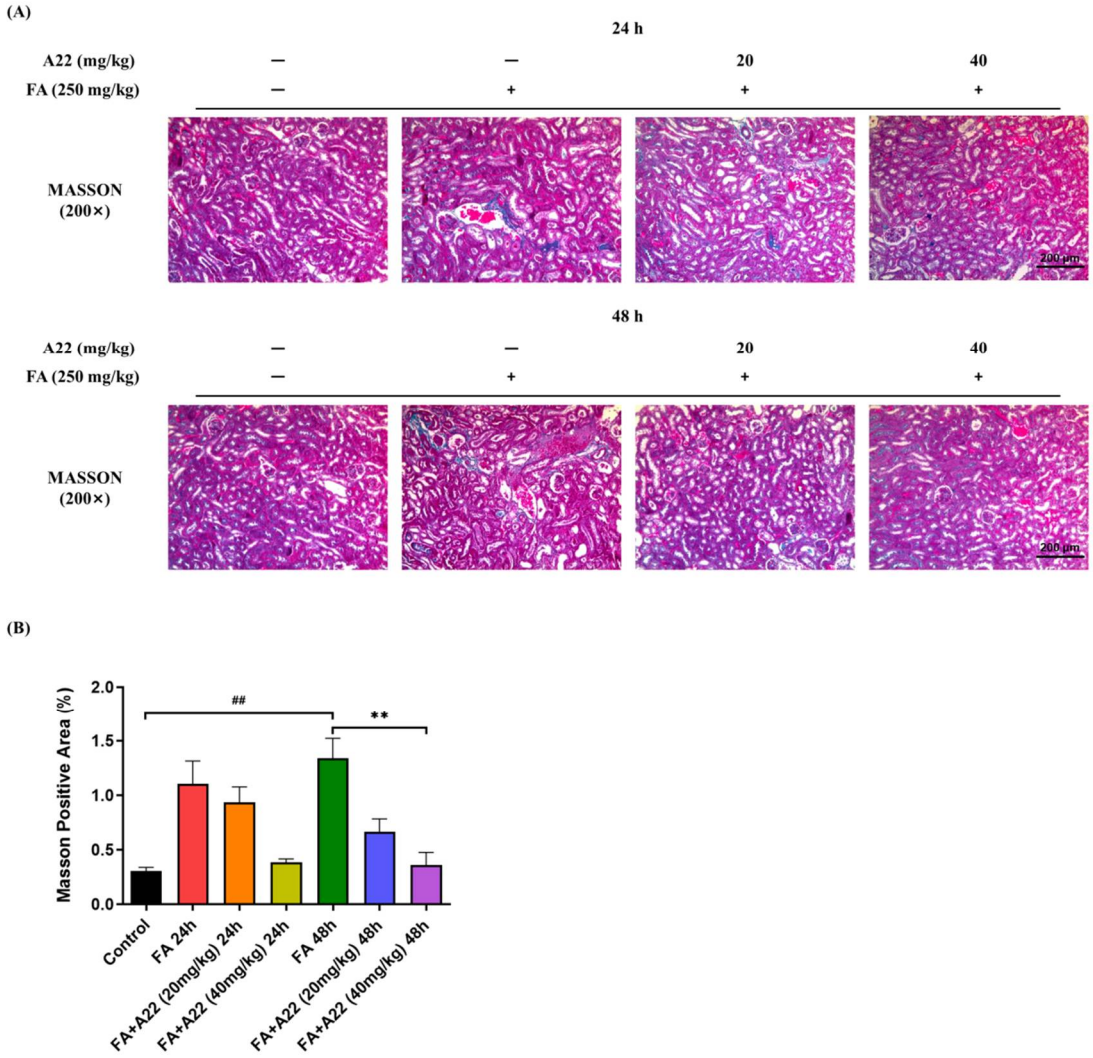


**Figure S2**. MASSON staining of mice kidney tissue showed that **A22** improved renal fibrosis in mice model. (A) Masson staining of the kidney tissues in mice (Magnification was 200x, and scale bar was 200 μm). As to kidney fibrosis, compared with control groups, the interstitial and perivascular collagen depositions were also obviously found in kidneys of FA groups. **A22** could alleviate collagen deposition of FA induced kidney injury tissues. Image J software was used to count the collagen deposition area, and **A22** could reduce collagen deposition area in a dose-dependent manner. Data were statistically analyzed as shown in (B) as mean ± SEM, with each column indicating one group. $^{\#\#}p<0.01$, vs. Control group; $^{**}p<0.01$, vs. FA 48h group.

## MATERIAL AND METHODS

### RNA extraction and qRT-PCR

Total RNA from cells and mice kidney was isolated by using RNAiso Plus (Takara, Cat# 9109, Japan). After homogenizing the sample, chloroform was added, and the homogenate was allowed to separate into a clear upper aqueous layer (containing RNA), an interface, and a red lower organic layer (containing the DNA and proteins). RNA was precipitated from the aqueous layer with isopropanol. DNA was precipitated from the aqueous/organic interface with ethanol. cDNA was synthesized with a One-Step RT-PCR Kit (Takara, Cat# 639503, Japan).

Quantitative real-time polymerase chain reaction (PCR) was carried out using 2×RealStar SYBR Mixture (GenStar, Cat# A301-10, Guangzhou, China). The results were analyzed on a LightCycler480 II real-time PCR system (ROCHE, USA) using the 2-ΔΔCt method. Primers were synthesized by Sangon Biotec (Guangzhou, China) with sequences listed as shown in Table S1. Actin was used as a loading control and relative mRNA levels were normalized to Actin.

### Western blotting

Cell samples were lysed in cold RIPA extraction buffer (Beyotime, Cat# P0013C, Chengdu, China) with an addition of protease inhibitors (Roche, Cat# 4693006001, Guangzhou, China). The extracted proteins were separated by using SDS-PAGE and transferred to a polyvinylidene difluoride membrane (Millipore, Guangzhou). After blocking with TBS/T (0.1%) containing 5% bovine serum albumin (BSA) for 25-30 min at room temperature, the membrane was incubated with different primary antibodies of hnRNP LL (Affinity Biosciences OH, Cat# AF6139, USA), by 1:1,000 dilution in 5% bovine serum albumin at 4 °C overnight. The membrane was washed with TBS/T for 4 x 10 min to remove unbound antibodies, and then incubated with HRP-conjugated secondary antibodies (Cell Signaling Technology, Cat# 7076 from mouse and Cat# 7074 from rabbit, China). Protein bands were visualized with an ECL kit (Millipore, Cat# 64-201BP, China). Densitometry analysis was performed using Image J Software (Bio-Rad Laboratories, CA, USA) relative to the loading control.

The types of primary antibodies were BCL-2 (affinity biosciences OH, Cat# AF6139, USA), BAX (affinity biosciences OH, Cat# AF0120, USA), Cyto-c (affinity biosciences OH, Cat# AF0146, USA), Caspase9 (affinity biosciences OH, Cat# AF6348, USA), cleaved-Caspase9 (affinity biosciences OH, Cat# AF5240, USA), Caspase3 (affinity biosciences OH, Cat# AF6311, USA), cleaved-Caspase3 (affinity biosciences OH, Cat# AF7022, USA), P-PERK (Cell Signaling Technology, Cat# 3192S, USA), P-eIF-2α (Cell Signaling Technology, Cat# 3298T, USA), CHOP (Cell Signaling Technology, Cat# 2895T, USA), IL-6 (Cell Signaling

Technology Cat# 12153, USA), TNF-α (Cell Signaling Technology Cat# 6945, USA), IL-1β (Cell Signaling Technology Cat# 6956, USA)