Supplementary information

The Role of Activating Transcription Factor 3 in Metformin's Alleviation of Gastrointestinal Injury Induced by Restraint Stress in Mice

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Running title: Metformin protects against gastrointestinal injury through ATF3

Key words: Metformin, restraint stress, gastrointestinal injury, gastrointestinal leakage, gastrointestinal epithelial cell, apoptosis, activating transcription factor 3, tight junction

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Figure S1

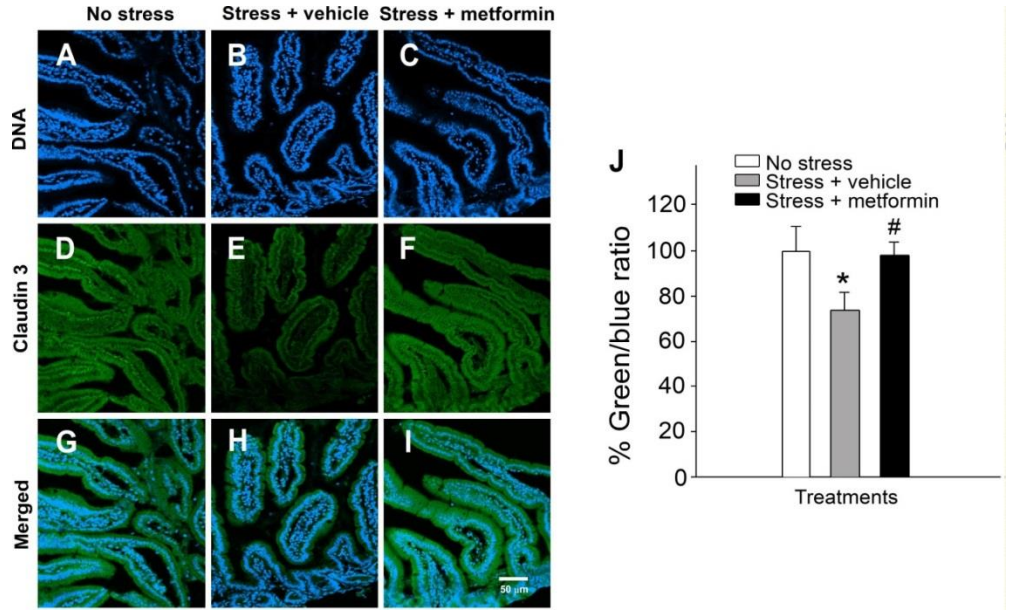


Figure S1. Immunohistochemistry (IHC) staining of GI tight junction protein claudin 3 (CLDN3) visualized through confocal microscopy. (A-I) Representative IHC images of mouse duodenal tissues are displayed. (A-C) DAPI staining shows DNA signals. (D-F) Signals derived from anti-CLDN3 antibody staining. (G-I) Merged signals display DNA and CLDN3. (A, D, G) Images derived from the no stress groups; (B, E, H) images from stress groups treated with vehicle; (C, F, I) Images from stress groups treated with metformin. (J) Quantitative results derived from three images per condition. * $P < 0.05$, compared to the no stress groups; # $P < 0.05$, compared to the vehicle-treated groups. N = 3. To quantify CLDN3 expression, in the merged images, the mean fluorescence intensity of CLDN3 (green) was normalized by dividing it with the mean fluorescence intensity of DNA (blue), which is proportional to the cell number. The average green/blue signal ratio of the no stress groups was normalized to 100 %. Scale bar: 50 µm.

Table S1

List of primers used in the qRT-PCR analysis of this report

Gene symbol	Forward primer (5'→3')	Reverse primer (5'→3')	Product size (bp)	Temperature (°C)
GAPDH	TCAACAGCAACTCCCACTCTTCCA	ACCCTGTTGCTGTAGCCGTATTCA	115	55
PRKAA1 (AMPK)	GAAACCTGAGAACGTCCTGC	GCCTGCGTACAATCTTCCTG	157	54
ATF3	AGTGACAGCATGAGCCCTCT	GCAGCACTGACCTGATCAAA	179	60
HIF1-α	CCAGCAGACCCAGTTACAGA	TGAGTGCCACTGTATGCTGA	186	55
NRF 2	GAGCTAGATAGTGCCCCTGG	CAGGACTCACGGGAACTTCT	169	52
PIK3CA	CTTGACCTTCGCATGCTACC	AGCCATTGATGCAGTGTGTG	158	54
AKT 1	AAGGACGGTGCCACTATGAA	TCCTGGTTGTAGAAGGGCAG	158	55