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## Supplemental information

# A tRNA-derived fragment of ginseng protects heart against ischemia/reperfusion injury via targeting the lncRNA MIAT/VEGFA pathway

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## Table S1. Top 10 candidate targets prioritized by bioinformatics prediction.

| Gene Symbol | Type | Context+ | Structure score | Energy | [a]Associated disease |
|---|---|---|---|---|---|
| Pclo | Non-Coding | -0.303 | 314 | -38.66 | Pontocerebellar hypoplasia |
| Pi16 | Non-Coding | -0.396 | 290 | -34.75 | Corneal dystrophy<br>Prostate cancer |
| Atp2b4 | Coding | -0.341 | 286 | -31.67 | Long Qt Syndrome |
| P2rx2 | Coding | -0.535 | 286 | -28.29 | Deafness |
| Actr5 | Coding | -0.264 | 179 | -30.52 | Intellectual disability |
| Ovol1 | Coding | -0.122 | 172 | -32.05 | Colon Small Cell Carcinoma<br>Fetal Encasement Syndrome. |
| Spryd3 | Coding | -0.229 | 171 | -34.77 | Lissencephaly |
| Ccdc28a | Coding | -0.203 | 171 | -26.33 | Bardet-Biedl Syndrome |
| MIAT | Non-Coding | -0.038 | 170 | -30.1 | Myocardial infarction<br>Nervous system disease. |
| Pdzd3 | Coding | -0.28 | 169 | -27.6 | Diarrhea |

[a]Referred to GeneCard (https://www.genecards.org/)

## Table S2. Sequence of the biotinylated DNA probes for purification of ginseng tRNAs.

| tRNA | Probe sequence (5'-Biotin) | Length (mer) | Mass (Da) | Tm (℃) |
|---|---|---|---|---|
| $tRNA^{His(GUG)}$ | **5'**-CATGGTGGATTCACAATCCACTGCCTTGAT-3' | 30 | 9564.0 | 68 |
| $tRNA^{Gly(GCC)}$ | **5'**-TCCTTGGCAAAGAGAAATTTTACCATTCGA-3' | 30 | 9596.1 | 64 |
| $tRNA^{Met(CAU)}$ | **5'**-TGACCTCAAGGTTATGAGCCTTGCGAGCTA-3' | 30 | 9629.1 | 70 |
| $tRNA^{Leu(UAA)}$ | **5'**-ACGCTCTTTAGCACGAGATTTTGAGTCTCG-3' | 30 | 9595.50 | 68 |

## Table S3. Sequence information of tRFs derived from ginseng.

| Source | Code | Antisense derived from ginseng's tRNA (5' to 3') | Sense (5' to 3') | Length (mer) | Group |
|---|---|---|---|---|---|
| $tRNA^{His(GUG)}$ | HC70 | GCGGAUGUAGCCAAGUGGAUCA | UGAUCCACUUGGCUACAUCCGC | 22 | 5'-tRF |
| | HC71 | UCAAUUCCCGUCGUUCGCCCCA | UGGGGCGAACGACGGGAAUUGA | 22 | 3'-tRF |
| $tRNA^{Asp(GUC)}$ | HC72 | GGGAUUGUAGUUCAAUCGGUCA | UGACCGAUUGAACUACAAUCCC | 22 | 5'-tRF |
| | HC73 | UCGAGCCCCGUCAGUCCCGCCA | UGGCGGGACUGACGGGGCUCGA | 22 | 3'-tRF |
| $tRNA^{Gly(GCC)}$ | HC74 | GCGGAUAUAGUCGAAUGGUAAA | UUUACCAUUCGACUAUAUCCGC | 22 | 5'-tRF |
| | HC75 | UCGAUUCCCGCUAUCCGCCCCA | UGGGGCGGAUAGCGGGAAUCGA | 22 | 3'-tRF |
| $tRNA^{Leu(CAA)}$ | HC76 | GCCUUGGUGGUGAAAUGGUAGA | UCUACCAUUUCACCACCAAGGC | 22 | 5'-tRF |
| | HC77 | UCGAGUCCUCUUCAAGGCACCA | UGGUGCCUUGAAGAGGACUCGA | 22 | 3'-tRF |
| $tRNA^{Met(CAU)}$ | HC78 | CGCGGAGUAGAGCAGUUUGGUA | UACCAAACUGCUCUACUCCGCG | 22 | 5'-tRF |
| | HC79 | UCAAAUCCUGUCUCCGCAACCA | UGGUUGCGGAGACAGGAUUUGA | 22 | 3'-tRF |
| $tRNA^{Ser(GCU)}$ | HC80 | GGAGAGAUGGCUGAGUGGACUA | UAGUCCACUCAGCCAUCUCUCC | 22 | 5'-tRF |
| | HC81 | GGAGAGAUGGCUGAGUGGACUA | UGGCGGAAAGAGAGGGAUUCGA | 22 | 3'-tRF |
| $tRNA^{Gln(UUG)}$ | HC82 | UGGGGCGUGGCCAAGUGGUAAG | CUUACCACUUGGCCACGCCCCA | 22 | 5'-tRF |
| | HC83 | UCGAAUCCUUCCGUCCCAGCCA | UGGCUGGGACGGAAGGAUUCGA | 22 | 3'-tRF |
| $tRNA^{Glu(UUC)}$ | HC84 | GCCCCCAUCGUCUAGUGGUUCA | UGAACCACUAGACGAUGGGGGC | 22 | 5'-tRF |
| | HC85 | UCGACUUCCCCUGGGGGUACCA | UGGUACCCCCAGGGGAAGUCGA | 22 | 3'-tRF |
| $tRNA^{Asn(GUU)}$ | HC86 | UCCUCAGUAGCUCAGUGGUAGA | UCUACCACUGAGCUACUGAGGA | 22 | 5'-tRF |
| | HC87 | UCGAAUCCUACCUGGGGAGCCA | UGGCUCCCCAGGUAGGAUUCGA | 22 | 3'-tRF |
| $tRNA^{Pro(UGG)}$ | HC88 | AGGGAUGUAGCGCAGCUUGGUA | UACCAAGCUGCGCUACAUCCCU | 22 | 5'-tRF |
| | HC89 | GGUUCAAAUCCUGUCAUCCCUA | UAGGGAUGACAGGAUUUGAACC | 22 | 3'-tRF |

## Table S4. Sequence of primers used for quantitative real-time PCR analysis.

| Targeted RNA name | Primers (5'>3') | | Amplification efficiency |
|---|---|---|---|
| MIAT | Forward | **5'**-TGCAAGTCCCCTCGTCTCTA-3' | 104.2% |
| | Reverse | **5'**-AGACCAAAGGTAGCCACTGC-3' | |
| VEGFA | Forward | **5'**-CAGCTATTGCCGTCCAATTGA-3' | 95.3% |
| | Reverse | **5'**-CCAGGGCTTCATCATTGCA-3' | |
| $\beta$-action | Forward | **5'**-AAGATCAAGATCATTGCTCCTCC-3' | 91.8% |
| | Reverse | **5'**-TAACAGTCCGCCTAGAAGCA-3' | |

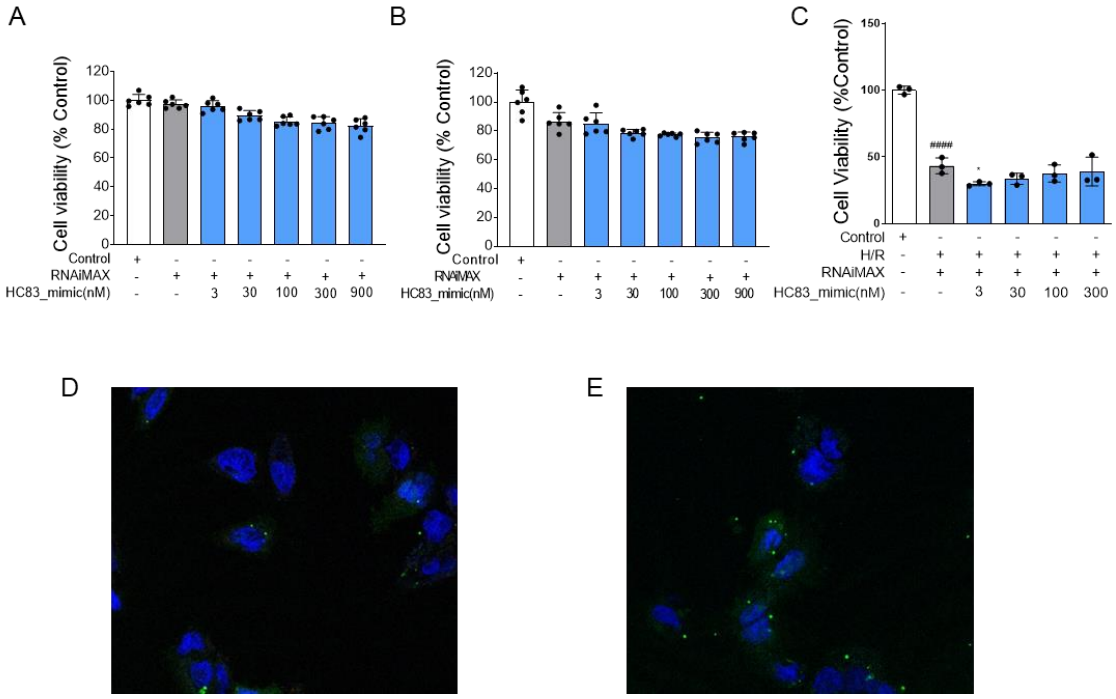


**Figure S1. Cell viability assay and confocal visualization of HC83_mimc transfected with Lipofectamine RNAiMAX Reagent in normal and H/R-injured H9c2 cells.** Cell viability assay of HC83_mimic at 3-900 nM transfected with Lipofectamine RNAiMAX Reagent for 24 hr (**A**) and 48 hr (**B**) in normal H9c2 cells by using CCK-8 kit. (**C**) Cell viability assay of HC83_mimic at 30-300 nM transfected with Lipofectamine RNAiMAX Reagent in H/R-injured H9c2 cells by using CCK-8 kit. (**D**) Fluorescence imaging of normal H9c2 cells which were treated with FAM-labeled HC83_mimic (green) at a concentration of 300 nM. Images were acquired by using a confocal laser microscope. Nuclei were stained with Hoechst 33342 (blue). (**E**) Fluorescence imaging of H/R-injured H9c2 cells which were treated with FAM-labeled HC83_mimic (green) at a concentration of 300 nM transfected with Lipofectamine RNAiMAX Reagent. Images were acquired by using a confocal laser microscope. Nuclei were stained with Hoechst 33342 (blue). Data are shown as the mean±SD. $^{\#\#\#\#}P$ <0.0001 *vs.* Control; $^{****}P$ <0.0001 *vs.* H/R, $^{***}P$ <0.001 *vs.* H/R, $^{**}P$ <0.01 *vs.* H/R, $^{*}P$ <0.05 *vs.* H/R. *P* values were calculated using One-way ANOVA.

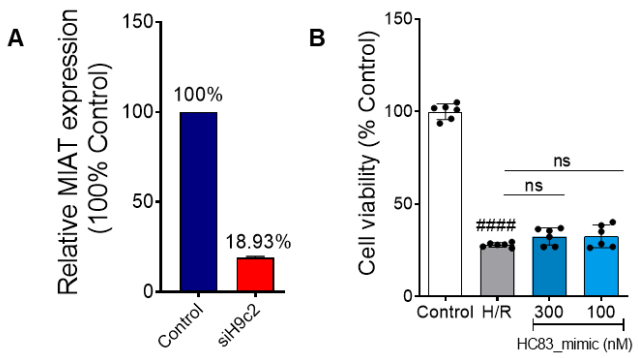


**Figure S2. Effects of HC83_mimic on H9c2 cells treated with siMIAT were abolished.** (**A**) The RNA expression level of MIAT on normal or H9c2 cells treated with siMIAT (siH9c2). H9c2 cells were treated with 300 nM siMIAT, then total RNA was extracted, and qRT-PCR was performed to evaluate the RNA expression level of MIAT. The results showed that the RNA expression level of MIAT of siH9c2 cells (18.93%) was significantly down-regulated compared to normal H9c2 cells (100%). (**B**) Cell viability assay of HC83_mimic on siH9c2 cells. The results showed that the effects of HC83_mimic on cell proliferation were almost abolished on siH9c2 cells. Data are shown as the mean±SD, $n = 6$. $^{####}P < 0.0001$ *vs.* Control. *P* values were calculated using One-way ANOVA.

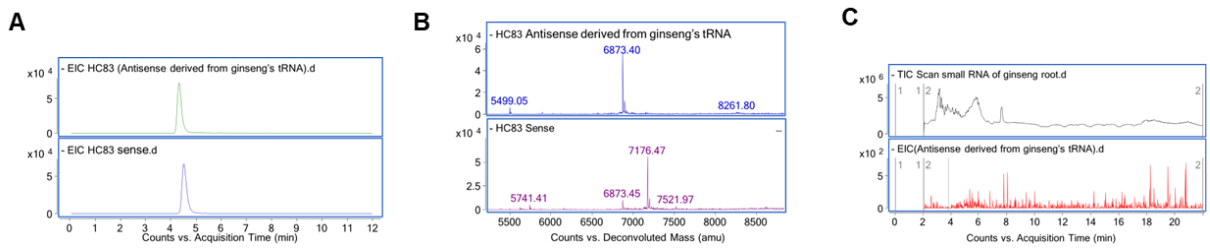


**Figure S3. Analysis of HC83 in small RNA of ginseng root by using the UHPLC-Q/TOF-MS method.** (**A-B**) The EIC and deconvolution MS spectra of synthetic HC83 (upper) and its complementary sequence (below). (**C**) The TIC of small RNA of ginseng root and EIC of HC83 from ginseng. HC83 from ginseng was lower than 0.5 ng in small RNA of ginseng root. It is too low to be detected.

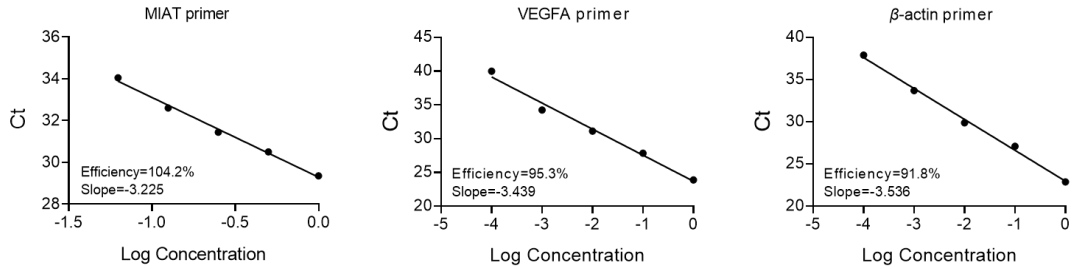


**Figure S4. Amplification efficiency detection of the primers used for qRT-PCR experiments.** For each PCR run, a standard curve of 5 independent dilutions was subjected. The calibration curves were constructed by plotting the Ct value against Log concentration, and the PCR amplification efficiency was calculated using the formula: $PCR\ efficiency = 10^{-1/slope} - 1$.