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Supplemental Information

mRNA Therapy Improves Metabolic and Behavioral Abnormalities in a Murine Model of Citrin Deficiency

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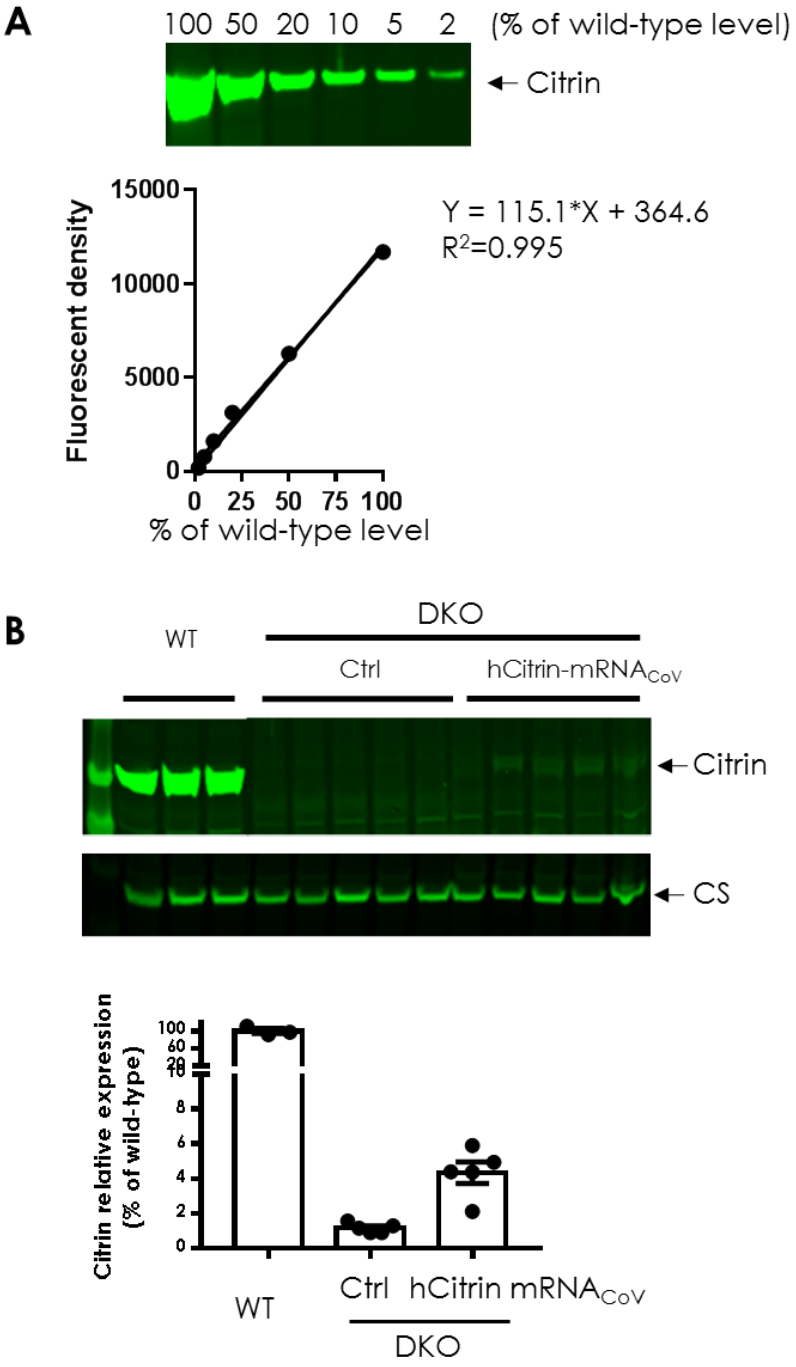
SUPPLEMENTARY FIGURES



Supplementary Fig. 1. hCitrin protein levels in citrin deficient mice (*Ctrn*$^{-/-}$). A) Immunoblot of increasing amounts of citrin protein from wild-type mouse liver mitochondrial extracts for generating citrin protein standard curve. The quantitative fluorescent signals from the citrin bands were plotted against the percentage of citrin protein expression level in wild-type mice,

showing a wide range of linearity. **B)** Citrin protein expression levels in citrin deficient mice (*Ctrn*$^{-/-}$) after repeat injections of hCitrn-mRNA. *Ctrn/mGPD*-dKO mice received three weekly injections (*i.v.*) of either control (Ctrl) or hCitrin-mRNA$_{CoV}$ at 0.5 mg/kg. Mice were euthanized at 24 hr after the third and final injection. Citrin protein expression levels in mitochondria prepared from mouse livers were assessed by Western blot analysis, normalized to Citrate Synthase (CS), a mitochondrial marker, and expressed as percentage of the average of wild-type group. The average hCitrin protein expression level introduced to the *Ctrn/mGPD*-dKO was 4.3% of wild-type level in mice. Citrin and CS protein expression levels were quantified using the Odyssey CLx instrument and accompanying software. WT, wild-type mice. Bars represent mean ± SEM (n = 3 – 5).

SUPPLEMENTARY TABLES

Supplementary Table 1. Complete mRNA sequence of hCitrin-mRNA$_{CoV}$. U = 5-methoxyuridine. UTR sequences are underlined.

GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGAGCCACCAUGGCCGCCGCCAAGGU
CGCCCUCACCAAGAGGGCGGACCCCGCGGAGCUCAGGACCAUCUUUCUCAAGUACGCCUCCAUCG
AGAAGAACGGGGAGUUCUUCAUGAGCCCGAACGAUUUCGUCACCAGGUACCUCAACAUCUUCGG
CGAGAGCCAGCCCAACCCGAAGACCGUAGAGCUCCUCAGCGGCGUCGUCGACCAGACGAAGGACG
GACUCAUUAGCUUCCAGGAGUUCGUCGCCUUCGAGAGCGUCCUCUGCGCGCCCGACGCCCUCUU
CAUGGUCGCGUUCCAGCUGUUCGAUAAGGCCGGCAAGGGCGAGGUGACCUUUGAAGAUGUGAA
GCAGGUGUUCGGUCAGACCACCAUCCACCAGCACAUCCCUUUUAACUGGGACUCAGAAUUCGUC
CAGCUGCACUUCGGCAAGGAAAGGAAGCGCCAUCUGACCUACGCCGAGUUCACACAGUUUCUCC
UGGAGAUCCAGCUGGAACACGCCAAACAGGCGUUCGUUCAGCGGGACAACGCCAGGACCGGCCG
GGUCACCGCGAUCGACUUCCGUGAUAUCAUGGUGACCAUCAGGCCUCACGUCCUGACGCCCUUC
GUGGAAGAGUGCCUGGUGGCCGCCGCGGGUGGGACCACGUCCCACCAGGUGAGCUUCUCCUAU
UUUAACGGGUUCAACAGCCUCCUCAACAACAUGGAGCUGAUCCGGAAGAUCUAUAGCACCCUGG
CCGGGACGCGCAAGGACGUCGAGGUGACCAAGGAAGAAUUCGUGCUGGCCGCCCAGAAGUUUG
GCCAGGUGACGCCCAUGGAGGUGGACAUCCUGUUCCAACUGGCGGACCUGUACGAACCCAGGGG
CCGGAUGACCCUGGCCGACAUCGAGCGCAUUGCCCCGCUCGAGGAGGGCACCCUGCCCUUCAACC
UGGCGGAGGCCCAGCGCCAGAAGGCCAGCGGGGACAGCGCCAGGCCCGUCCUGCUGCAGGUGGC
CGAGUCCGCGUACAGGUUCGGUCUGGGGAGCGUCGCGGGAGCCGUGGGUGCCACCGCCGUGUA
UCCGAUCGACCUCGUGAAGACCCGUAUGCAGAAUCAGAGGUCCACCGGCAGCUUCGUGGGCGAG
CUCAUGUACAAGAACUCCUUCGACUGUUUCAAGAAGGUUCUCCGGUACGAGGGGUUCUUCGGC
CUCUACCGGGGACUGCUACCCCAGCUGCUCGGCGUGGCGCCCGAGAAGGCCAUCAAGCUCACGG
UCAACGACUUUGUGCGGGACAAGUUCAUGCACAAGGACGGCAGCGUCCCACUGGCCGCCGAGAU
UCUGGCAGGCGGCUGCGCCGGAGGCAGCCAGGUCAUCUUUACCAACCCACUGGAGAUCGUGAAG
AUCAGGCUGCAAGUGGCCGGGGAGAUUACCACCGGACCCCGUGUGAGCGCCCUGAGCGUGGUG
AGGGACCUUGGCUUCUUUGGCAUAUACAAGGGAGCCAAGGCCUGCUUUCUGAGGGACAUCCCG
UUUUCCGCCAUAUACUUUCCCUGCUACGCCCACGUGAAAGCGAGCUUCGCCAACGAGGACGGCC
AGGUCAGUCCCGGCUCCCUACUGCUGGCCGGAGCCAUCGCCGGGAUGCCCGCCGCCAGCCUGGU
GACGCCCGCGGACGUUAUCAAGACCAGGCUGCAGGUGGCGGCCAGGGCGGGCCAGACCACCUAC
AGCGGCGUGAUCGACUGCUUCCGCAAGAUCCUGCGGGAGGAGGGGCCCAAGGCCCUGUGGAAA
GGCGCCGGGGCAAGGGUGUUCAGGAGCAGCCCACAGUUCGGGGUGACCCUGCUUACCUACGAAC
UGCUGCAGCGGUGGUUCUAUAUCGACUUCGGAGGAGUCAAGCCCAUGGGCUCCGAGCCUGUGC
CCAAGAGCAGGAUCAAUCUGCCCGCGCCCAACCCAGACCACGUGGGAGGGUACAAACUGGCCGUG
GCCACCUUCGCCGGAAUCGAGAACAAGUUCGGCCUGUAUCUGCCCCUGUUCAAACCGUCCGUCU
CCACCAGCAAGGCCAUCGGCGGAGGCCCCUGAUAAUAGUCCAUAAAGUAGGAAACACUACAGCU
GGAGCCUCGGUGGCCAUGCUUCUUGCCCCUUGGGCCUCCCCCCAGCCCCUCCUCCCCUUCCUGCA
CCCGUACCCCCCGCAUUAUUACUCACGGUACGAGUGGUCUUUGAAUAAAGUCUGAGUGGGCGG
CAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAUCUAG

Supplementary Table 2. Complete mRNA sequence of eGFP. U = N1m-pseudouridine. UTR sequences are underlined.

GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGAGCCACCAUGGUGUCCAAGGGUG
AGGAAUUGUUUACCGGGGUGGUGCCUAUUCUCGUCGAACUUGACGGGGAUGUGAAUGGACAC
AAGUUUUCGGUAUCCGGAGAAGGAGAGGGUGACGCCACAUACGGAAAGCUUACACUCAAAUUC
AUCUGUACGACGGGGAAACUGCCCGUACCCUGGCCUACGCUCGUAACCACGCUGACUUAUGGAG
UGCAGUGCUUUAGCAGAUACCCCGACCAUAUGAAGCAGCACGACUUCUUCAAGUCGGCGAUGCC
CGAGGGGUACGUGCAAGAGAGGACCAUUUUCUUCAAAGACGAUGGCAAUUACAAAACACGCGCA
GAAGUCAAGUUUGAGGGCGAUACUCUGGUCAAUCGGAUCGAAUUGAAGGGAAUCGAUUUCAAA
GAAGAUGGAAACAUCCUUGGCCAUAAGCUCGAGUACAACUAUAACUCGCAUAAUGUCUAUAUCA
UGGCUGACAAGCAGAAAAACGGUAUCAAAGUCAACUUUAAGAUCCGACACAAUAUUGAGGACGG
UUCGGUGCAGCUUGCGGACCACUAUCAACAGAAUACGCCGAUUGGGGAUGGUCCGGUCCUUUU
GCCGGAUAACCAUUAUCUCUCAACCCAGUCAGCCCUGAGCAAAGAUCCAAACGAGAAGAGGGACC
ACAUGGUCUUGCUCGAAUUCGUGACAGCGGCAGGGAUCACUCUGGGAAUGGACGAGUUGUACA
AGUGAUAAUAGGCUGGAGCCUCGGUGGCCAUGCUUCUUGCCCCUUGGGCCUCCCCCCAGCCCCU
CCUCCCCUUCCUGCACCCGUACCCCCGUGGUCUUUGAAUAAAGUCUGAGUGGGCGGCAAAAAAA
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