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Fatty liver disease protective *MTARC1* p.A165T variant reduces the protein stability of MTARC1

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Abstract

Non-alcoholic fatty liver disease (NAFLD) is one of the most common causes of liver disease worldwide. MTARC1, encoded by the *MTARC1* gene, is a mitochondrial outer membrane-anchored enzyme. Interestingly, the *MTARC1* p.A165T (rs2642438) variant is associated with a decreased risk of NAFLD, indicating that MTARC1 might be an effective target. It has been reported that the rs2642438 variant does not have altered enzymatic activity so we reasoned that this variation may affect MTARC1 stability. In this study, *MTARC1* mutants were generated and stability was assessed using a protein stability reporter system both *in vitro* and *in vivo*. We found that the *MTARC1* p.A165T variant has dramatically reduced the stability of MTARC1, as assessed in several cell lines. In mice, the MTARC1 A168T mutant, the equivalent of human MTARC1 A165T, had diminished stability in mouse liver. Additionally, several MTARC1 A165 mutants, including A165S, A165 N, A165V, A165G, and A165D, had dramatically decreased stability as well, suggesting that the alanine residue of MTARC1 165 site is essential for MTARC1 protein stability. Collectively, our data indicates that the *MTARC1* p.A165T variant (rs2642438) leads to reduced stability of MTARC1. Given that carriers of rs2642438 show a decreased risk of NAFLD, the findings herein support the notion that MTARC1 inhibition may be a therapeutic target to combat NAFLD.

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CRediT authorship contribution statement

Mengyue Wu: Investigation. **Meng Tie:** Investigation. **Liwei Hu:** Investigation. **Yunzhi Yang:** Writing – review & editing. **Yong Chen:** Writing – review & editing. **Daniel Ferguson:** Writing – review & editing. **Yali Chen:** Investigation, Funding acquisition. **Anyuan He:** Writing – review & editing, Writing – original draft, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrc.2024.149655.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is one of the most prominent causes of liver disease worldwide, affecting both adults and children. NAFLD will likely emerge as the leading cause of end-stage liver disease in the coming decades [1], but there are currently no pharmacological treatments available. To discover new therapeutic options, there is an urgent need to understand the molecular mechanisms driving the progression of NAFLD. The mitochondrial amidoxime-reducing component (MTARC1), a Mo (molybdenum)-containing enzyme encoded by the *MTARC1* gene, is involved in the activation of N-hydroxylated prodrugs [2–4]. However, the physiological role of MTARC1 remains unknown.

Interestingly, *MTARC1* p.A165T (rs2642438), a variant with 29 % of minor allele frequency (MAF), is associated with a decreased risk of NAFLD [5–9]. Since *MTARC1* p.A165T (rs2642438) does not alter the enzymatic activity of MTARC1 [10], we reasoned that this variant may affect the stability of this protein. Herein, we developed a sensitive reporter system to explore the effect of several different mutations on MTARC1 stability both *in vitro* and *in vivo*. Using this reporter, we discovered that MTARC1 A165T has reduced stability. Furthermore, substituting alanine 165 for other amino acids with different features also led to a decrease in MTARC1 stability as well, suggesting that the alanine residue of this site is critical for its protein stability. Given that the *MTARC1* p.A165T (rs2642438) variant is highly associated with a decreased risk of NAFLD, our findings support the notion that the inhibition of MTARC1 could be targeted therapeutically to combat NAFLD.

2. Materials and methods

2.1. Cell culture

HEK293T, HepG2, Li-7, LO2, and Huh-7 cells were maintained in DMEM supplemented with 10 % fetal bovine serum. To obtain stable cell lines, cells of interest were infected with lentivirus and selected with puromycin at the dosage of 2 μg/mL for 72 h.

2.2. Virus packaging

Adeno-associated virus serotype 8 packaging and production were performed by OBiO Technology (Shanghai). Lentivirus was packaged as described previously [11]. Briefly, HEK293T cells were transfected with pMD2.G, psPAX2, and transfer plasmid, and the medium containing the virus was harvested at 72 h post-transfection.

2.3. Plasmids

Plasmid pLJM1-GFP (Addgene #19319) was digested with AgeI and EcoRI. Then the fragment of HA-P2A-GFP prepared by PCR was cloned into the position of GFP by using an in-fusion cloning strategy following the instruction provided in the kit (Vazyme, C115) to generate a backbone plasmid pLJM1-EcoRI-HA-P2A-GFP. Wild-type (WT) *MTARC1* ORF was prepared by PCR using cDNA from human cell line HepG2 and cloned into the site of EcoRI of pLJM1-EcoRI-HA-P2A-GFP using in-fusion cloning

to generate plasmid pLJM1-MTARC1-HA-P2A-GFP. For each mutant of MTARC1, two fragments were prepared by PCR using the WT *MTARC1* as a template and cloned into pLJM1-EcoRI-HA-P2A-GFP with the same cloning strategy as described above. Cre of AAV.TBG. PI.Cre.rBG plasmid (Addgene #107787) was replaced by HA-P2A-GFP to make a backbone plasmid AAV.TBG.HindIII.HA.P2A.GFP using the strategy as reported previously [12]. Mouse *Mtarc1* was amplified by PCR using cDNA from the mouse liver as the template and then cloned to the HindIII site of AAV.TBG.HindIII.HA.P2A.GFP to make a plasmid AAV.TBG.MTARC1.HA.P2A.GFP. For mouse *Mtarc1* p.A168T, two fragments were prepared by PCR using the WT *Mtarc1* as a template and cloned into pLJM1-EcoRI-HA-P2A-GFP with the same cloning strategy as described above.

2.4. Animal experiments

Animal experiments were performed following the guidelines provided by the Ethics Committees of Anhui Medical University. C57BL/6J male mice were purchased from GemPharmatech (Nanjing, China). All mice had free access to water and food (normal chow diet) and were housed in a specific pathogen-free environment with a 12-h light/12-h dark cycle. Adeno-associated virus serotype 8 (AAV8), suspended in 200 μL phosphate-buffered saline (PBS), was injected via the tail vein at the dosage of $2e^{11}$ viral genome/mouse. Mice were sacrificed for liver collection at two weeks post-virus administration.

2.5. Immunoblotting and immunoprecipitation

Cells and liver tissues were subjected to protein extraction as described previously [12,13]. Briefly, cells and liver tissues were lysed with RIPA buffer supplemented with a proteinase inhibitor cocktail, homogenized using a homogenizer, and then centrifuged at 12,000 rpm for 10 min. The supernatant was collected and subjected to bicinchoninic acid (BCA) assay. Forty μg of total proteins for each sample were loaded for SDS-PAGE with a standard protocol. Immunoprecipitation was performed as described previously [13]. Briefly, harvested cells were lysed with a lysis buffer (Tris-HCl 20 mM, NaCl 100 mM, EDTA 1 mM, Glycerol 10 %, SDS 0.2 %, TritonX-100 0.5 %, NP-40 0.1 %). The lysate was centrifuged at 12,000 rpm for 10 min, and then the supernatant was transferred to a new tube, and subjected to the preclear operation using anti-mouse nanobody agarose beads and immunoprecipitation using anti-HA nanobody agarose beads. Antibodies are listed below: HA (51064–2-AP), GFP (50430–2-AP), Vinculin (66305–1-Ig), Ubiquitin (10201–2-AP), Tubulin (11224–1-AP), anti-mouse HRP (SA00001–1), and anti-Rabbit HRP (SA00001–2) from Proteintech (China); anti-mouse nanobody agarose beads (KTSM1341), and anti-HA nanobody agarose beads (KTSM1305) from AlpaLifeBio (China). The quantification of blotting bands was performed using ImageJ.

3. Results

3.1. MTARC1 p.A165T promotes the degradation of MTARC1

Given that *MTARC1* p.A165T (rs2642438) does not alter the enzymatic activity of MTARC1 [10], we reasoned that *MTARC1* p.A165T may affect the stability of MTARC1. To test this hypothesis, we linked MTARC1 and GFP with the P2A element—a “self-cleaving” peptide [14], which makes MTARC1 and GFP have a fixed ratio of protein

synthesis. They are “cleaved” once they are synthesized so that GFP can act as an internal reference to compare the stability of MTARC1 variants (Fig. 1A). We named this method the Protein Stability Reporter System (PSRS). Utilizing PSRS, we found that *MTARC1* p.A165T had dramatically reduced the stability of MTARC1 as assessed in multiple cell lines (Fig. 1B, C, 1D, 1E, 1F, and 1G; Figs. S1A and S1B). To confirm the conclusion derived from PSRS, we chased HepG2 cells stably expressing *MTARC1* variants with cycloheximide—an inhibitor of mRNA translation [15] and found that the half-life of MTARC1 A165T is much shorter than the wild-type protein (3.5 vs 11.5 h) (Fig. 1H and I). In line with this observation, MARC1 A165T is highly ubiquitinated (Fig. 1J). Interestingly, *MTARC1* p.M187K, a raw variant associated with a decreased risk of NAFLD as well [16], does not affect protein stability (Figs. S1C, S1D, S1E, and S1F), indicating *MTARC1* p.M187K might affect the enzymatic activity of MTARC1. Taken together, *MTARC1* p. A165T (rs2642438) promotes the degradation of MTARC1.

3.2. Mouse Mtarc1 p.A168T, the counterpart of human MTARC1 p. A165T, promotes the degradation of MTARC1

Mouse MTARC1 A168T is the counterpart of human MTARC1 A165T (Fig. 2A). To confirm the findings above *in vivo*, we coupled the PSRS with adeno-associated virus serotype 8 (AAV8) to compare the protein stability of different variants in mouse liver (Fig. 2B). In line with our *in vitro* findings, mouse MTARC1 A168T shows remarkably reduced stability compared to the wild type protein as assessed in mouse liver (Fig. 2C and D), indicating that MTARC1 p.A165T variant modulates the stability of MTARC1 in an evolutionarily conserved manner.

3.3. Alanine residue of MTARC1 site 165 is essential for MTARC1 protein stability

Next, we explored how *MTARC1* p.A165T increases MTARC1 degradation. The β-position of threonine is a hydroxyl group. Compared to the methyl group of alanine, the hydroxyl group of threonine is hydrophilic. To mimic this feature, we generated an MTARC1 A165S mutant that also showed reduced stability as assessed in HEK293T cell (Fig. 3A and 3B). Of note, both threonine (T) and serine (S) can be phosphorylated. To test whether the capability of phosphorylation of this site could manipulate its stability, MTARC1 A165D, a phosphorylation mimetic mutant, was generated and showed reduced stability as well (Fig. 3A and 3B). Surprisingly, MTARC1 A165V, a dephosphorylation mimetic mutant, showed reduced stability also (Fig. 3A and 3B), suggesting that the capability of phosphorylation of MTARC1 A165T does not affect MTARC1 stability. Additionally, both MTARC1 A165G and A165 N exhibited reduced stability as well (Fig. 3A and 3B). The findings above were further confirmed in HepG2 cells (Fig. 3C and D). Taken together, our data suggests that the alanine residue of MTARC1 site 165 is essential for MTARC1 protein stability.

4. Discussion

MTARC1 p.A165T (rs2642438), a newly identified variant with 29 % of MAF, is significantly associated with lower ALT levels, lower hepatic fat, and lower all-cause liver cirrhosis [6,8,16]. These findings suggest that MTARC1 could be a potential therapeutic target for liver disease. However, the mechanism underlying this seemingly protective

variant remains unknown. Interestingly, Ott et al. found that both the wild-type (WT) and p.A165T variant of *MTARC1* expressed in *E.coli* present comparable binding ability of its cofactor molybdenum [10]. Furthermore, MTARC1 A165T showed no distinguished kinetic parameters in the N-reduction of benzamidoxime [10], suggesting that *MTARC1* p.A165T might not alter the enzymatic activity of MTARC1.

In this study, we found that *MTARC1* p.A165T robustly promotes the degradation of MTARC1, implying that the loss/inhibition of MTARC1 could prevent liver disease progression. Of note, Hudert et al. found that MTARC1 is expressed at similar levels across rs2642438G > A genotypes in a relatively small cohort, as assessed by proteomics, although they predicted that the variant *MTARC1* p.A165T could exert a destabilizing effect [7]. Since the information on *MTARC1* messenger is absent, it's not feasible to assess the stability of MTARC1 variants in that work [7]. Interestingly, it seems that the alanine residue of the MTARC1 165 site is critical for protein stability since replacing alanine with several other amino acids promotes the degradation of MTARC1 as well, implying that the substitution of this amino acid might lead to the misfolding of the MTARC1 protein followed by degradation. However, how the p.165 mutant of MTARC1 is degraded requires further study.

Notably, no cases of liver cirrhosis are observed in a small cohort of carriers of *MTARC1* p.R200Ter—a rare truncated variant with 0.009 % of MAF [6]. Since this observation derives from a small cohort and the carriers are heterozygous, it might not be appropriate to conclude that the loss of MTARC1 could lead to the protective effect of liver disease. Of note, *MTARC1* p.M187K, with 1.1 % of MAF, is also associated with a reduced risk of liver disease, similar to *MTARC1* p.A165T [16]. Our data suggests that *MTARC1* p.A165T, but not p.M187K, promotes the degradation of MTARC1 protein, implying these two variants cause the same phenotype, most likely via two different mechanisms. Given that the former promotes the degradation of MTARC1, the latter might inhibit the enzymatic activity of MTARC1. While this work was in progress, Lewis et al. found that hepatocyte-specific *MTARC1* knockdown alleviated diet-induced hepatic fat accumulation, but not fibrosis [17]. Given the findings of Lewis [17], our results here suggest that the deficiency of MTARC1 in non-hepatocytes might contribute to the decreased risk of all-cause fibrosis/cirrhosis associated with *MTARC1* p. A165T.

In summary, our data suggests that *MTARC1* p.A165T but not p. M187K promotes the degradation of MTARC1 protein. Given that *MTARC1* p.A165T with 29 % of MAF is highly associated with a reduced risk of liver disease, our data support the notion that the inhibition of MTARC1 could be targeted therapeutically to treat liver diseases such as fibrosis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data availability statement

Further information and requests for resources, and reagents should be directed to, and will be fulfilled by, the lead contact, AH (heanyuan85@foxmail.com).

Abbreviations

NAFLD	Non-alcoholic fatty liver disease
MTARC1	Mitochondrial Amidoxime Reducing Component 1
AAV8	Adeno-associated virus serotype 8
IP	immunoprecipitation
MAF	minor allele frequency
PSRS	Protein Stability Reporter System

References

[1]. Younossi Z, Anstee QM, Marietti M, Hardy T, Henry L, Eslam M, George J, Bugianesi E, Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention, Nature reviews, Gastroenterol. Hepatol 15 (2018) 11–20.

[2]. Gruenewald S, Wahl B, Bittner F, Hungeling H, Kanzow S, Kotthaus J, Schwering U, Mendel RR, Clement B, The fourth molybdenum containing enzyme mARC: cloning and involvement in the activation of N-hydroxylated prodrugs, J. Med. Chem 51 (2008) 8173–8177. [PubMed: 19053771]

[3]. Klein JM, Busch JD, Potting C, Baker MJ, Langer T, Schwarz G, The mitochondrial amidoxime-reducing component (mARC1) is a novel signal-anchored protein of the outer mitochondrial membrane, J. Biol. Chem 287 (2012) 42795–42803. [PubMed: 23086957]

[4]. Kubitza C, Bittner F, Ginsel C, Havemeyer A, Clement B, Scheidig AJ, Crystal structure of human mARC1 reveals its exceptional position among eukaryotic molybdenum enzymes, Proc. Natl. Acad. Sci. U.S.A 115 (2018) 11958–11963. [PubMed: 30397129]

[5]. Innes H, Buch S, Hutchinson S, Guha IN, Morling JR, Barnes E, Irving W, Forrest E, Pedergnana V, Goldberg D, Aspinall E, Barclay S, Hayes PC, Dillon J, Nischalke HD, Lutz P, Spengler U, Fischer J, Berg T, Brosch M, Eyer F, Datz C, Mueller S, Peccerella T, Deltenre P, Marot A, Soyka M, McQuillin A, Morgan MY, Hampe J, Stickel F, Genome-Wide association study for alcohol-related cirrhosis identifies risk loci in MARC1 and HNRNPUL1, Gastroenterology 159 (2020) 1276, 1289.e1277. [PubMed: 32561361]

[6]. Luukkonen PK, Juuti A, Sammalkorpi H, Penttilä AK, Orešič M, Hyötyläinen T, Arola J, Orho-Melander M, Yki-Järvinen H, MARC1 variant rs2642438 increases hepatic phosphatidylcholines and decreases severity of non-alcoholic fatty liver disease in humans, J. Hepatol 73 (2020) 725–726. [PubMed: 32471727]

[7]. Hudert CA, Adams LA, Alisi A, Anstee QM, Crudele A, Draijer LG, Furse S, Hengstler JG, Jenkins B, Karnebeek K, Kelly DA, Koot BG, Koulman A, Meierhofer D, Melton PE, Mori TA, Snowden SG, van Mourik I, Vreugdenhil A, Wiegand S, Mann JP, Variants in mitochondrial amidoxime reducing component 1 and hydroxysteroid 17-beta dehydrogenase 13 reduce severity

of nonalcoholic fatty liver disease in children and suppress fibrotic pathways through distinct mechanisms, Hepatol. Commun 6 (2022) 1934–1948. [PubMed: 35411667]

[8]. Kalinowski P, Smyk W, Nowosad M, Paluszkiewicz R, Michałowski Ł, Ziarkiewicz-Wróblewska B, Weber SN, Milkiewicz P, Lammert F, Zieniewicz K, Krawczyk M, MTARC1 and HSD17B13 variants have protective effects on non-alcoholic fatty liver disease in patients undergoing bariatric surgery, Int. J. Mol. Sci 23 (2022).

[9]. Chen Y, Du X, Kuppa A, Feitosa MF, Bielak LF, O'Connell JR, Musani SK, Guo X, Kahali B, Chen VL, Smith AV, Ryan KA, Eirksdottir G, Allison MA, Bowden DW, Budoff MJ, Carr JJ, Chen YI, Taylor KD, Oliveri A, Correa A, Crudup BF, Kardia SLR, Mosley TH Jr, Norris JM, Terry JG, Rotter JI, Wagenknecht LE, Halligan BD, Young KA, Hokanson JE, Washko GR, Gudnason V, Province MA, Peyser PA, Palmer ND, Speliotes EK, Genome-wide association meta-analysis identifies 17 loci associated with nonalcoholic fatty liver disease, Nat. Genet 55 (2023) 1640–1650. [PubMed: 37709864]

[10]. Ott G, Reichmann D, Boerger C, Cascorbi I, Bittner F, Mendel RR, Kunze T, Clement B, Havemeyer A, Functional characterization of protein variants encoded by nonsynonymous single nucleotide polymorphisms in MARC1 and MARC2 in healthy Caucasians, Drug Metabol. Dispos.: Biol. Fate Chem 42 (2014) 718–725.

[11]. Park H, He A, Tan M, Johnson JM, Dean JM, Pietka TA, Chen Y, Zhang X, Hsu FF, Razani B, Funai K, Lodhi IJ, Peroxisome-derived lipids regulate adipose thermogenesis by mediating cold-induced mitochondrial fission, J. Clin. Invest 129 (2019) 694–711. [PubMed: 30511960]

[12]. Han B, Chen Y, Song C, Chen Y, Chen Y, Ferguson D, Yang Y, He A, Autophagy modulates the stability of Wee1 and cell cycle G2/M transition, Biochem. Biophys. Res. Commun 677 (2023) 63–69. [PubMed: 37549603]

[13]. He A, Chen X, Tan M, Chen Y, Lu D, Zhang X, Dean JM, Razani B, Lodhi IJ, Acetyl-CoA derived from hepatic peroxisomal β-oxidation inhibits autophagy and promotes steatosis via mTORC1 activation, Mol. Cell 79 (2020) 30–42.e34. [PubMed: 32473093]

[14]. Ryan MD, King AM, Thomas GP, Cleavage of foot-and-mouth disease virus polyprotein is mediated by residues located within a 19 amino acid sequence, J. Gen. Virol 72 (Pt 11) (1991) 2727–2732. [PubMed: 1658199]

[15]. Schneider-Poetsch T, Ju J, Eyler DE, Dang Y, Bhat S, Merrick WC, Green R, Shen B, Liu JO, Inhibition of eukaryotic translation elongation by cycloheximide and lactimidomycin, Nat. Chem. Biol 6 (2010) 209–217. [PubMed: 20118940]

[16]. Emdin CA, Haas ME, Khera AV, Aragam K, Chaffin M, Klarin D, Hindy G, Jiang L, Wei WQ, Feng Q, Karjalainen J, Havulinna A, Kiiskinen T, Bick A, Ardissino D, Wilson JG, Schunkert H, McPherson R, Watkins H, Elosua R, Bown MJ, Samani NJ, Baber U, Erdmann J, Gupta N, Danesh J, Saleheen D, Chang KM, Vujkovic M, Voight B, Damrauer S, Lynch J, Kaplan D, Serper M, Tsao P, Mercader J, Hanis C, Daly M, Denny J, Gabriel S, Kathiresan S, A missense variant in Mitochondrial Amidoxime Reducing Component 1 gene and protection against liver disease, PLoS Genet. 16 (2020) e1008629. [PubMed: 32282858]

[17]. Lewis LC, Chen L, Hameed LS, Kitchen RR, Maroteau C, Nagarajan SR, Norlin J, Daly CE, Szczerbinska I, Hjuler ST, Patel R, Livingstone EJ, Durrant TN, Wondimu E, BasuRay S, Chandran A, Lee WH, Hu S, Gilboa B, Grandi ME, Toledo EM, Erikat AHA, Hodson L, Haynes WG, Pursell NW, Coppieters K, Fleckner J, Howson JMM, Andersen B, Ruby MA, Hepatocyte mARC1 promotes fatty liver disease, JHEP Rep.: Innovat. Hepatol 5 (2023) 100693.

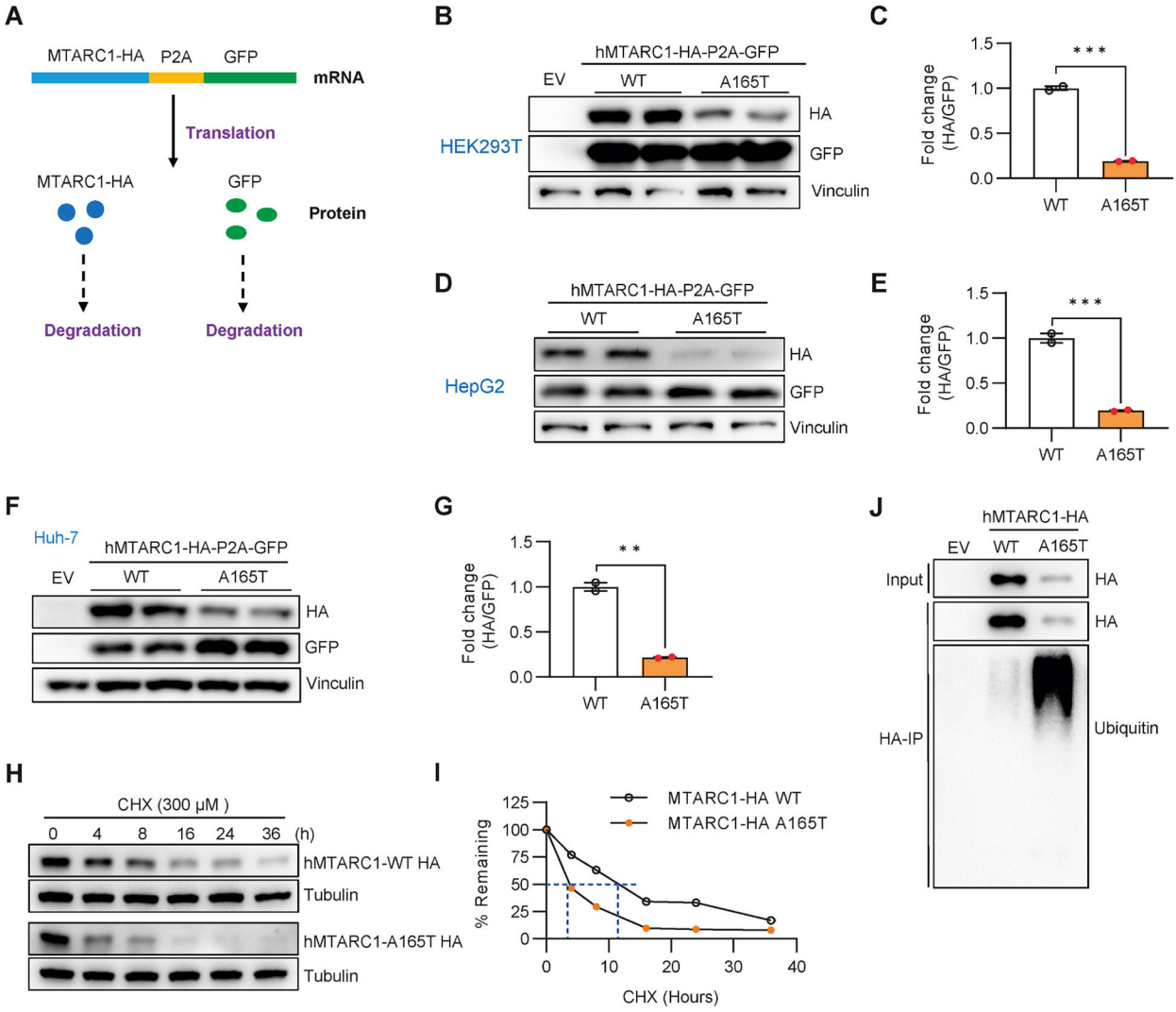

Fig. 1.

MTARC1 p.A165T variant reduces the stability of MTARC1. (A) The graphic view of the Protein Stability Reporter System. HA: HA tag; P2A: P2A element—a "self-cleaving" peptide. (B–C) HEK293T, (D–E) HepG2, and (F–G) Huh-7 cells stably expressing MTARC1-HA-P2A-GFP were subjected to immunoblotting and quantification (n = 2/group). (H–I) HepG2 cells stably expressing MTARC1-HA wild-type (WT) or A165T variant were subjected to cycloheximide chase (300 μM), and harvested for immunoblotting and quantification at the indicated time. The half-life of MTARC1 WT and A165T are 11.5 and 3.5 h, respectively. (J) HEK293T cells stably expressing MTARC1-HA WT and A165T were subjected to immunoprecipitation (IP) using an anti-HA antibody followed by immunoblotting. Data were expressed as mean ± SEM and analyzed by Student's t-test. ***$p < 0.001$.

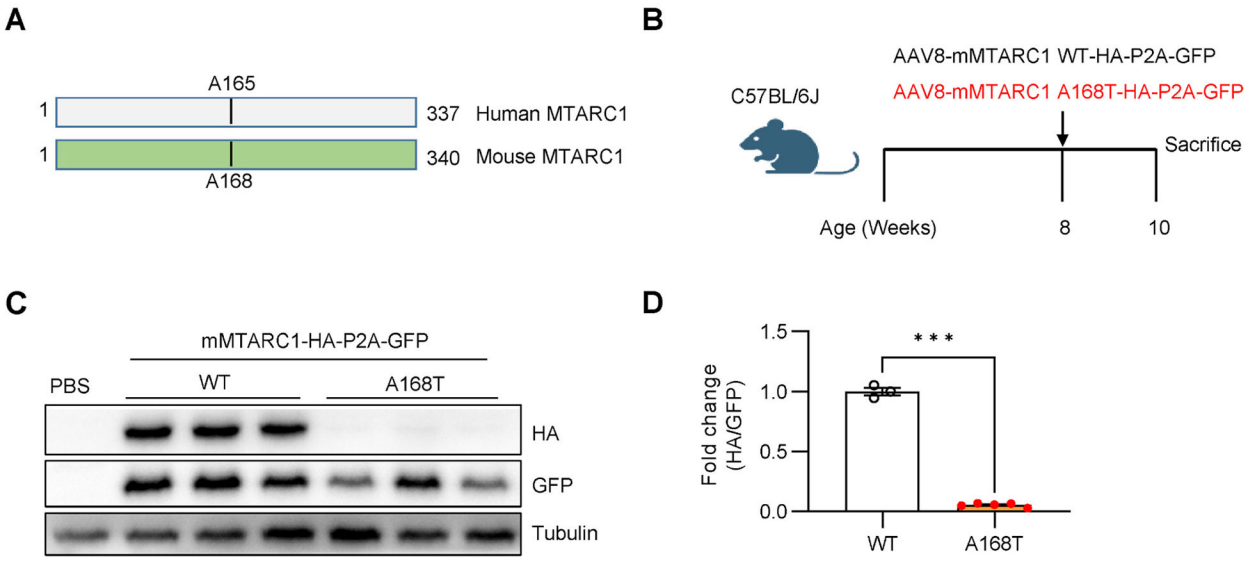

Fig. 2.

Mouse *Mtarc1* p.A168T variant, the counterpart of human *MTARC1* p.A165T, reduces the stability of MTARC1. (A) The graphic view showing the alignment of human and mouse MTARC1 protein. (B) The workflow of the experiment comparing protein stability of mouse MTARC1 WT and A168T variant *in vivo*. (C–D) Liver samples from (B) were subjected to immunoblotting and quantification (n = 3–5/group). Data were expressed as mean ± SEM and analyzed by Student's t-test. ***$p < 0.001$.

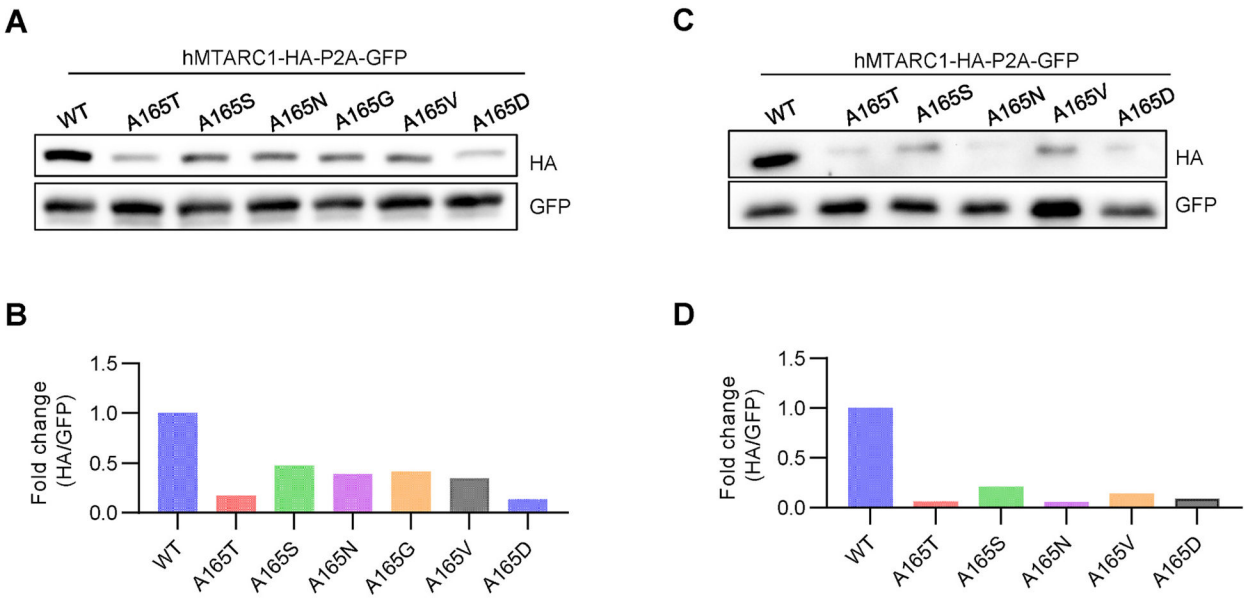

Fig. 3.

Alanine residue of MTARC1 site 165 is essential for MTARC1 protein stability. (A–B) HEK293T and (C–D) HepG2 cells stably expressing the indicated MTARC1 variants were subjected to immunoblotting and quantification.