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DELE1 is protective for mitochondrial cardiomyopathy

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Abstract

Mitochondrial dysfunction in heart triggers an integrated stress response (ISR) through phosphorylation of eIF2α and subsequent ATF4 activation. DAP3 Binding Cell Death Enhancer 1 (DELE1) is a mitochondrial protein recently found to be critical for mediating mitochondrial stress-triggered ISR (MSR)-induced eIF2α-ATF4 pathway activation. However, the specific role of DELE1 in heart at baseline or in response to mitochondrial stress remains largely unknown. In this study, we report that DELE1 is dispensable for cardiac development and function under baseline conditions. Conversely, DELE1 is essential for mediating an adaptive response to mitochondrial dysfunction-triggered stress in the heart, playing a protective role in mitochondrial cardiomyopathy.

Keywords

Mitochondrial cardiomyopathy; Dele1; Mitochondrial stress; Integrated stress response

1. Introduction

Mitochondria are known as the powerhouse of the cardiomyocyte, providing over 90% of ATP required for normal heart function. Mitochondria also regulate metabolism of amino acids, lipids, and iron, redox and calcium homeostasis, and apoptosis in cardiomyocytes [1]. Mutations in nuclear or mitochondrial DNA encoding crucial mitochondrial proteins

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Declaration of Competing Interest

None.

Appendix A. Supplementary data

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frequently result in mitochondrial cardiomyopathies. Mitochondrial dysfunction also has been widely observed in heart failure regardless of etiology [1]. In addition to serving multiple key functions for the cell, mitochondria are increasingly recognized as a hub for cell signaling cascades in maintaining normal cellular functions and stress responses. Mitochondrial dysfunction in the heart triggers an integrated stress response (ISR) through the phosphorylation of eIF2α and activates ATF4, a master transcriptional regulator of the cellular stress response [2–6]. A CRISPR interference (CRISPRi) screen for proteins required for mitochondrial stress-triggered ISR (MSR)-induced ATF4 activation in cultured cells identified a novel protein, DAP3 Binding Cell Death Enhancer 1 (DELE1) [7,8]. DELE1 is a poorly characterized mitochondrial protein, first identified as a novel DAP3-binding protein crucial for death receptor-mediated apoptosis [9]. While it is clear that DELE1 mediates MSR-induced ATF4 activation, the specific role of DELE1 in heart at baseline or in response to mitochondrial stress remains largely unknown. In this study, we generated a *Dele1* floxed mouse model and used it to generate cardiomyocyte-specific *Dele1* knockout (cKO) mice. Results revealed that loss of DELE1 in cardiomyocytes does not cause cardiac abnormities up to one year at baseline. We further investigated DELE1 function in mitochondrial cardiomyopathy by crossing the *Dele1* knockout allele into fetal and adult mitochondrial cardiomyopathy models [10,11]. Results demonstrated that deletion of DELE1 was detrimental for both fetal and adult mitochondrial cardiomyopathy, demonstrating that DELE1-mediated MSR is a protective signaling pathway in mitochondrial cardiomyopathy.

2. Materials and methods

2.1. Mouse models

C57BL/6NCrl breeder mice (strain code: 027) were purchased from Charles River. The UCSD animal care personnel maintained all animals, and the IACUC of UCSD approved all experimental procedures. 100 mg/kg Ketamine and 5 mg/kg Xylazine was approved to use as primary anesthetic in terminal procedures (tissue harvest etc.), followed by cervical dislocation as the secondary euthanasia method. Both male and female mice were included in the experiment. The Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/CRISPR-associated 9 (Cas9) system was utilized to generate the *Dele1* floxed allele with exon 5 of the mouse *Dele1* gene flanked by 2 LoxP sites. *Dele1* cardiomyocyte-specific knockout (cKO) male (*Dele1*$^{f/f}$; Cre+) mice were generated by crossing *Dele1* floxed mice with *Xenopus laevis* myosin light- chain 2 (*Xmlc2*)-Cre males, which have been widely used to ablate genes in cardiomyocytes from embryonic day 7.5 with no cardiac toxicity [11]. Age and sex-matched homozygous *Dele1* floxed but Cre-negative littermates served as controls. Cardiomyocyte specific *Ptpmt1/Dele1* double knockout (dcKO) and *Taz/Dele1* dcKO mice were generated as described in our previous publication [6]. Polymerase chain reaction (PCR) primer sequences for genotyping are listed in Table II in the Data Supplement.

2.2. Statistical analyses

Data are presented as the mean ± SEM unless indicated otherwise. Statistical analysis was performed using GraphPad Prism 8.0 or SPSS software, with a 2-tailed Student *t*-test or

2-way ANOVA for comparisons among groups. The Bonferroni test was used for post-hoc pairwise comparisons. *P* values of <0.05 were considered statistically significant.

Additional details on the methods used in this study are available in the supplemental material.

3. Results and discussion

3.1. Dele1 cKO mice display normal cardiac function and morphology

To study the role of DELE1 in cardiomyocytes in vivo, we generated a floxed *Dele1* mouse line with exon 4 of the mouse *Dele1* gene flanked by two LoxP sites (Fig. 1A). We then generated cardiomyocyte-specific knockout (cKO) mice by crossing floxed *Dele1* mice with *Xmlc2*-Cre transgenic mice [11]. Quantitative real-time PCR (qPCR) by utilizing primers outside of the floxed region revealed that deletion of exon 4 caused *Dele1* mRNA decay in cKO hearts (Fig. 1B). *Dele1* cKO mice were born at expected Mendelian ratios and survived beyond one year of age. We observed no sudden death or premature lethality of *Dele1* cKO mice, demonstrating that loss of DELE1 did not affect survival. We next evaluated cardiac function in *Dele1* cKO and control mice over the course of one year by echocardiography (Fig. 1C–F). Cardiac function in *Dele1* cKO mice was comparable to Cre negative littermates (35.0% vs. 34.7% FS), as were LVIDd (both 3.75 vs 3.83 mm) and LVIDs (2.44 vs. 2.50 mm) and LVPWd (0.72 vs. 0.73 mm) at 55 weeks. We observed no significant change in any of these parameters irrespective of sex. In addition, morphological and histological analyses at 55 weeks of age revealed no evidence of morphological defects in *Dele1* cKO mice, compared with controls (Fig. 1G). There were also no changes in global indexes of cardiac hypertrophy as measured by the ratio of heart weight to body weight and heart weight to tibia length in *Dele1* cKO mice and wild-type control mice (Fig. 1H and I). We also observed no changes in expression levels of cardiac fetal gene markers atrial natriuretic factor (*Nppa*) and B-type natriuretic peptide (*Nppb*) as well as the profibrotic gene markers collagen a1 type I (*Coll1a1*) and type III (*Coll3a1*) (Fig. 1J and K). Together, these results revealed no evidence of cardiac dysfunction in *Dele1* cKO mice and demonstrated that DELE1 is not essential for murine cardiac development and function under baseline conditions.

3.2. Deletion of DELE1 is detrimental in both fetal and adult mitochondrial cardiomyopathy

Recent findings showed that DELE1 mediates MSR-induced ATF4 activation [7,8]. Knockdown of DELE1 in cultured cells attenuates MSR triggered eIF2α-ATF4 signaling. To investigate the role of DELE1 in the setting of fetal mitochondrial cardiomyopathy, we employed our previously published *Ptpmt1* cardiomyocyte-specific knockout (PKO) mouse model as a fetal mitochondrial cardiomyopathy model [10]. PTPMT1 is a phosphatase encoded by nuclear DNA and exclusively localized to the mitochondria [12]. Deletion of PTPMT1 in mouse embryonic fibroblasts [12], mouse embryonic stem cells [13] or cardiomyocytes [10] leads to severe mitochondrial dysfunction and malformation. Loss of PTPMT1 in cardiomyocytes results in abnormal cardiac development starting at embryonic day (E) 12.5, and embryonic lethality at E18.5 [10]. Our previous study revealed that

deletion of PTPMT1 in cardiomyocytes elevated ISR evident by eIF2 ns phosphorylation and increased ATF4 protein and its target genes [10]. We observed no increase in XBP1-or ATF6 target genes, negating the likelihood that endoplasmic reticulum stress (ER stress) and unfolded protein response (UPR) were involved in the activation of ISR in PTPMT1 deficient hearts [10]. We further demonstrated that the activation of ISR in PTPMT1 deficient hearts is not dependent on GCN2, which is the principal mediator of amino acid deprivation-induced ISR, by utilizing PKO/GCN2$^{-/-}$ double knockout (dKO) mice [10]. Moreover, loss of HRI, the critical eIF2α kinase mediated MSR [7,8], abolished the activation of eIF2α-ATF4 signaling in PTPMT1 deficient hearts [10]. The foregoing evidence indicates that activation of ISR in PKO hearts is owing to mitochondrial stress. Our *Ptpmt1* cKO mouse model provides us with a unique model to investigate the role of DELE1 in fetal mitochondrial cardiomyopathy *in vivo*, thus we crossed *Dele1* floxed alleles with *Ptpmt1* cardiomyocyte-specific knockout (PKO) mice. We found that all the cardiomyocyte-specific *Ptpmt1/Dele1* double knockout mice (dcKO) died at E16.5, while a majority of PKO mice survived at this stage with abnormal heart morphology, suggesting that loss of DELE1 negatively impacted survival of embryos with PKO fetal mitochondrial cardiomyopathy (Fig. 2A-B). Our qRT-PCR and western blot analyses revealed that, consistent with results in cultured cells [7,8], deletion of DELE1 abolished activation of eIF2α-ATF4 signaling in response to the MSR in PKO hearts (Fig. 2C-D). These results demonstrated that DELE1-mediated MSR-induced ATF4 activation is protective for fetal mitochondrial cardiomyopathy.

To determine the role of DELE1 in adult mitochondrial cardiomyopathy, we crossed *Dele1* floxed mice with our previously published *Tafazzin* (*Taz*) cardiomyocyte-specific knockout (TKO) mice, a model of adult mitochondrial cardiomyopathy [11]. *Taz* is a mitochondrial phospholipid-lysophospholipid acyltransferase, involved in the remodeling/maturation of cardiolipin (CL) [14]. Mutations in *TAZ* cause Barth syndrome, a X-linked cardiac and skeletal mitochondrial myopathy [14]. TKO mice displayed mitochondrial malformations and dysfunction at 2 months of age and dilated cardiomyopathy at 4 months of age. The majority of TKO mice can survive more than one year with impaired but stabilized cardiac function, suggesting the heart is adapting to mitochondrial dysfunction [11]. Western blot and qRT-PCR analysis confirmed that eIF2α-ATF4 signaling was activated in TKO hearts but abolished in *Taz/Dele1* dcKO hearts (Fig. 2E-F). Intriguingly, *Taz/Dele1* dcKO mice died between postnatal day (P) 10–12 with significantly enlarged hearts (Fig. 2G-H), compared to TKO mice that survive more than one year with cardiac dysfunction [11]. We also observed an increased ventricular weight to body weight ratio in dcKO, compared with TKO, *Dele1* cKO and wildtype controls (Fig. 2I). Echocardiographic analysis revealed severe cardiac dysfunction in dcKO mice (Fig. 2J-L). These results suggested that deletion of DELE1 is catastrophic for cardiomyopathy in TKO mice, demonstrating the beneficial role of DELE1-mediated MSR in adult mitochondrial cardiomyopathy.

Taken together, we report that DELE1 is likely dispensable for cardiac development and function at baseline conditions. Conversely, DELE1 is essential for mediating an adaptive response to mitochondrial dysfunction-triggered stress. Mitochondrial dysfunction elicits stress signaling through DELE1 and triggers an ISR pathway, a typical stress response pathway for eIF2α-ATF4 activation. Deletion of DELE1 was detrimental for both fetal and

adult cardiomyopathy. Our results, together with recent findings on deletion of other MSR components in the setting of mitochondrial cardiomyopathy [3–6], reveal a protective role for MSR in mitochondrial cardiomyopathy. During the preparation of this manuscript, Ahola et al. reported that global deletion of DELE1 worsens the cardiac phenotype of skeletal and cardiac muscle-specific *Cox10* knockout mice, and shortens lifespan of mutants from an average of 4 weeks to 3 weeks [15]. Acceleration of the cardiac phenotype in *Dele1/Cox10* double knockouts was attributed to increased ferroptosis [15], however, ferroptosis has yet to be examined in cardiac tissue. These data suggest that intention to completely inhibit the DELE1-eIF2α-ATF4 pathway in mitochondrial cardiomyopathy could be detrimental, rather than beneficial, for cardiac dysfunction. However, recent data suggested that knockdown of DELE1 in cultured cells triggers an alternative response in which specific molecular chaperones were induced under mitochondrial stress [8]. It remains unknown whether partially attenuating this pathway might be beneficial in the setting of mitochondrial cardiomyopathy. Notably, the current studies rely on gene knockout models of mitochondrial proteins. Whether PTPMT, TAZ, or CL are directly involved in the DELE1-mediated MSR at either molecular or functional levels remains to be addressed. Given that mitochondrial dysfunction also has been widely observed in heart failure regardless of etiology [1], it is of great interest to further investigate potential roles of DELE1 in acquired cardiomyopathies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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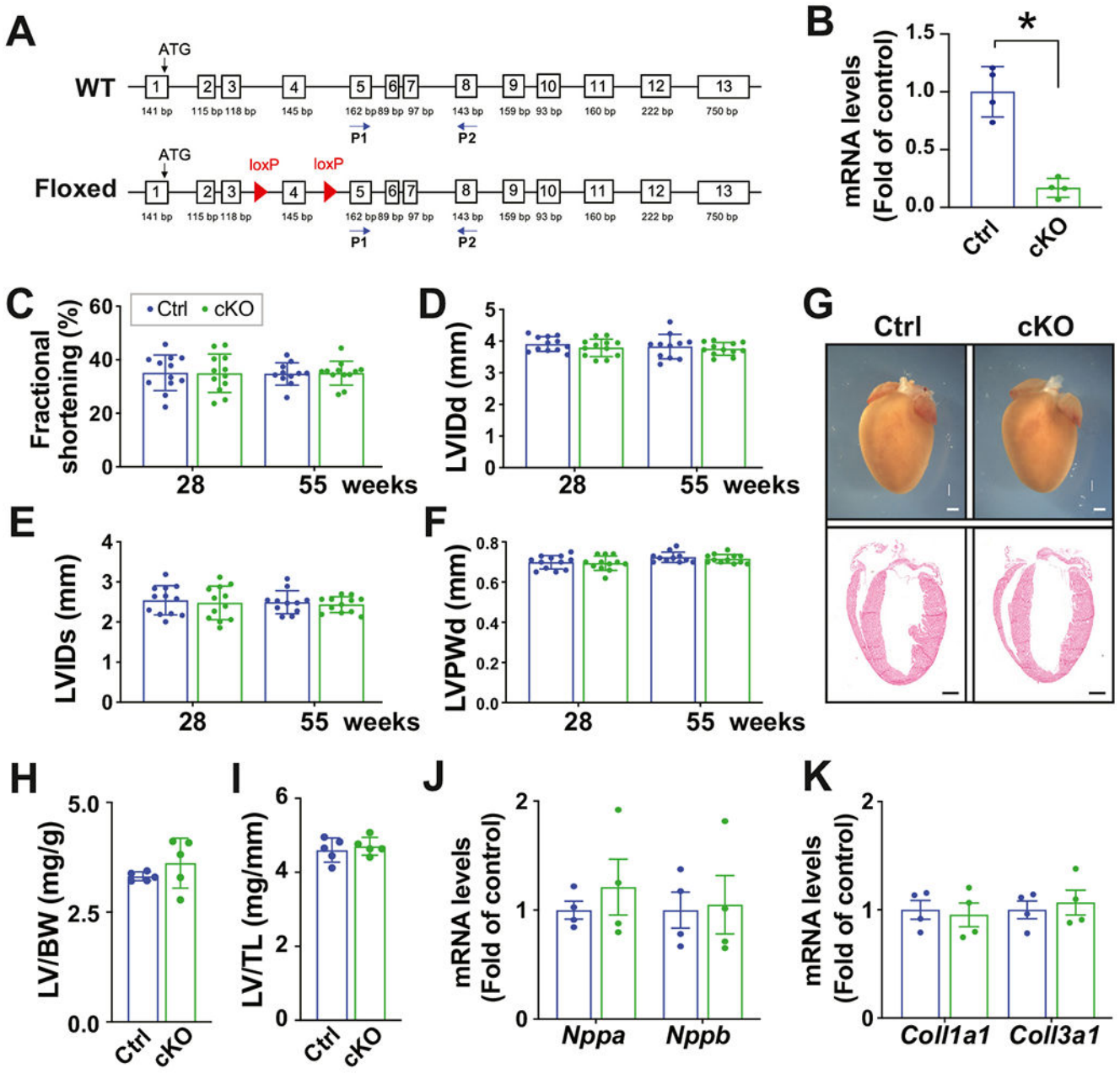

Fig. 1.

Dele1 cKO mice display normal cardiac function and morphology.

(A) Targeting strategy for the generation of *Dele1* floxed mice. Two of LoxP sites (red) flanking exon 4 of *Dele1* were inserted by utilizing CRISPR/Cas9 technology. Polymerase chain reaction (PCR) primer sets for mRNA quantitative real-time PCR (qRT-PCR) genotyping are indicated by blue arrows (P1 and P2). (B) qRT-PCR analysis of *Dele1* mRNA in *Dele1* cardiomyocyte-specific knockout (cKO) and control (Ctrl) mouse hearts at postnatal day 12. $n = 4$ mice per group. (C—F) Echocardiographic measurements for Ctrl and cKO male mice of (C) left ventricle (LV) percent fractional shortening (% FS), (D) end-diastolic LV internal diameter (LVIDd) and (E) end-systolic LV internal diameter

(LVIDs), as well as LV posterior wall thickness at the end-diastolic (LVPWd) at 28 and 55 weeks of age. $n = 12$ mice per group. (G) Representative microscopic views of whole mouse hearts (top) and 4-chamber sectional views of hematoxylin and eosin-stained sections (botton) from *Dele1* cKO and Ctrl mice at 55 weeks of age. Scale bar: 1 mm. (H—I) Ventricular weight (LV) to body weight (BW) ratio (H) and LV to tibia length (TL) ratio (I) for *Dele1* cKO versus Ctrl mice at 55 weeks of age. $n = 5$ mice per group. (J-K) qRTPCR analysis of cardiac fetal gene markers, atrial natriuretic factor (*Nppa*) and B-type natriuretic peptide (*Nppb*) (J), as well as collagen α1 type I (*Coll1a1*) and type III (*Coll3a1*) (K) in *Dele1* cKO and Ctrl mouse hearts at 55 weeks of age. $n = 4$ mice per group. Data were normalized to corresponding 18S levels, and cKO is expressed as the fold-change vs Ctrl. $^*P < 0.05$ by a 2-tailed Student *t*-test.

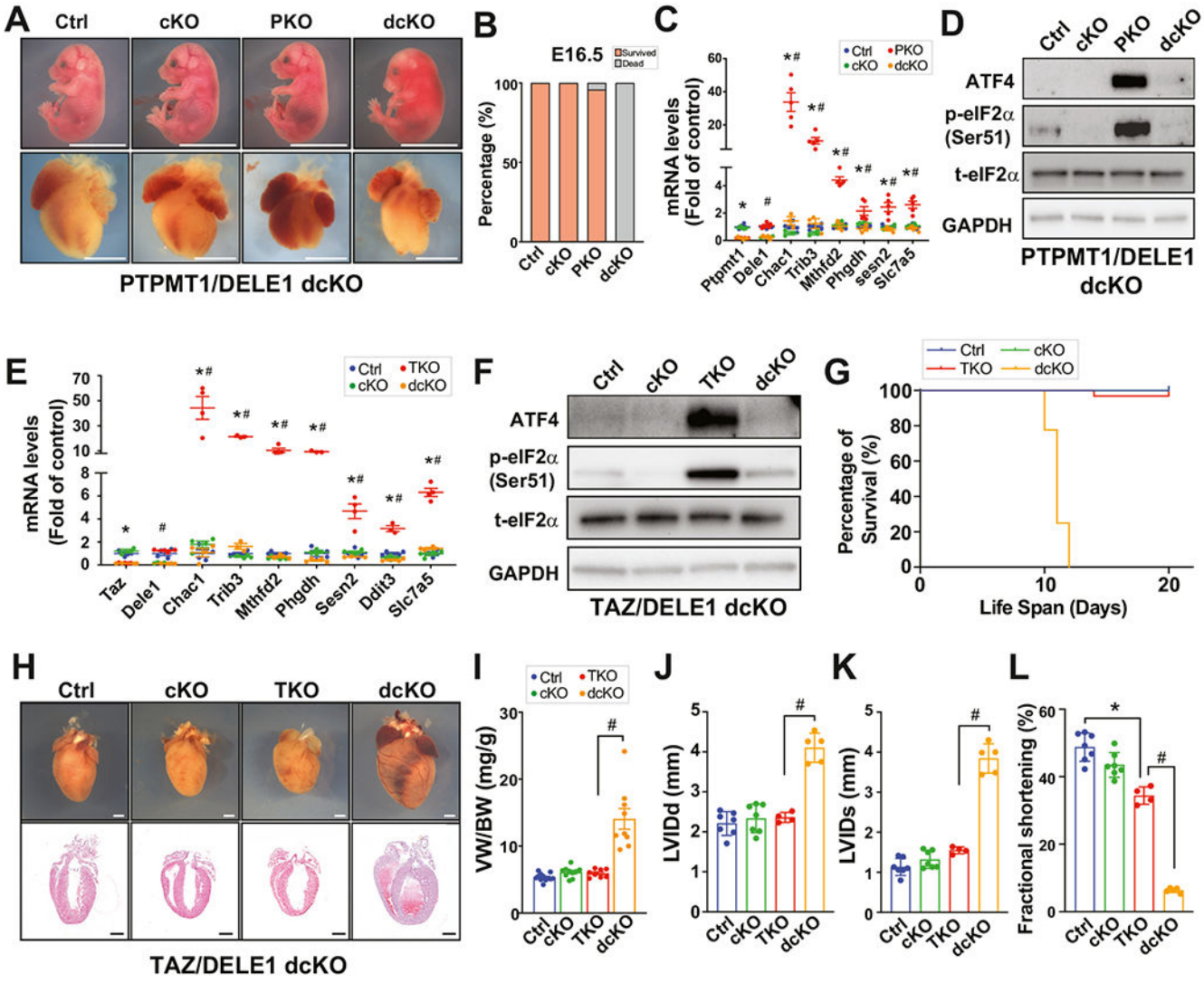

Fig. 2.

Deletion of DELE1 is detrimental in both fetal and adult mitochondrial cardiomyopathy. (A) Whole embryonic (top) and heart (bottom) morphology of Ctrl, *Dele1* cardiomyocyte-specific knockout (cKO), *Ptpmt1* cardiomyocyte-specific knockout (PKO) and cardiomyocyte-specific *Ptpmt1/Dele1* double knockout (dcKO) hearts at E16.5. Scale bar: 1 mm. n = 8–10 per group. (B) Percentage of survived (pink) and dead (grey) embryos in PKO and *Ptpmt1/Dele1* double knockout (dcKO). n = 17–21 per group. (C) qRT-PCR analysis of ATF4 target genes in Ctrl, cKO, PKO, and *Ptpmt1/Dele1* dcKO hearts at E11.5. n = 3–5 per group. (D) Western blot analysis of ATF4, total eIF2α (t-eIF2α), and phosphorylate eIF2α at Ser51 in Ctrl, cKO, PKO, and *Ptpmt1/Dele1* dcKO hearts at E11.5. n = 3 per group. (E) qRT-PCR analysis of ATF4 target genes in Ctrl, cKO, Taz cardiomyocyte-specific knockout (TKO) and cardiomyocyte-specific *Taz/Dele1* double knockout (dcKO) hearts at postnatal day (P) 10. n = 3–4 per group. (F) Western blot analysis of ATF4, total eIF2α (t-eIF2α) and phosphorylated eIF2α at Ser51 in Ctrl, cKO, TKO, and *Taz/Dele1* dcKO hearts at P10. n = 3 per group. (G) Kaplan-Meier survival curves for Ctrl,

cKO, TKO, and *Taz/Dele1* dcKO mice, $n = 33–36$ per group. (H) Whole-mount (top) and H&E stained sections (bottom) for Ctrl, cKO, TKO, and *Taz/Dele1* dcKO hearts at P10. Scale bar: 1 mm. n = 3 per group. (I) Cardiac ventricular weight (VW) to body weight (BW) ratio for Ctrl, cKO, TKO, and *Taz/Dele1* dcKO mice at P10. $n = 8–12$ per group. (J-L) Echocardiographic measurements of end-diastolic LV internal diameter (LVIDd) (J), end-systolic LV internal diameter (LVIDs) (K) and left ventricular percentage of fractional shortening (% FS) (L) for Ctrl, cKO, TKO, and *Taz/Dele1* dcKO mice at P10. $n = 4–7$ mice per group. GAPDH was used as a loading control for western blots. qRT-PCR data were normalized to corresponding 18S levels, and levels in mutants are expressed as the fold-change versus Ctrl. Data are represented as the mean ± SEM. $^{*}P < 0.05$ PKO, TKO, or dcKO vs. Ctrl, or as indicated; $^{\#}P < 0.05$ dcKO vs. PKO or TKO, or as indicated, by two-way ANOVA (four group comparison).