Stromal interaction molecule 1 is essential for normal cardiac homeostasis through modulation of ER and mitochondrial function

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Collins HE, He L, Zou L, Qu J, Zhou L, Litovsky SH, Yang Q, Young ME, Marchase RB, Chatham JC. Stromal interaction molecule 1 is essential for normal cardiac homeostasis through modulation of ER and mitochondrial function. Am J Physiol Heart Circ Physiol 306: H1231–H1239, 2014. First published February 28, 2014; doi:10.1152/ajpheart.00075.2014.—The endoplasmic reticulum (ER) Ca2+ sensor stromal interaction molecule 1 (STIM1) has been implicated as a key mediator of store-dependent and store-independent Ca2+ entry pathways and maintenance of ER structure. STIM1 is present in embryonic, neonatal, and adult cardiomyocytes and has been strongly implicated in hypertrophic signaling; however, the physiological role of STIM1 in the adult heart remains unknown. We, therefore, developed a novel cardiomyocyte-restricted STIM1 knockout (+/−STIM1-KO) mouse. In cardiomyocytes isolated from +/−STIM1-KO mice, STIM1 expression was reduced by −92% with no change in the expression of related store-operated Ca2+ entry proteins, STIM2, and Orai1. Immunoblot analyses revealed that +/−STIM1-KO hearts exhibited increased ER stress from 12 wk, as indicated by increased levels of the transcription factor C/EBP homologous protein (CHOP), one of the terminal markers of ER stress. Transmission electron microscopy revealed ER dilatation, mitochondrial disorganization, and increased numbers of smaller mitochondria in +/−STIM1-KO hearts, which was associated with increased mitochondrial fission. Using serial echocardiography and histological analyses, we observed a progressive decline in cardiac function in +/−STIM1-KO mice, starting at 20 wk of age, which was associated with marked left ventricular dilatation by 36 wk. In addition, we observed the presence of an inflammatory infiltrate and evidence of cardiac fibrosis from 20 wk in +/−STIM1-KO mice, which progressively worsened by 36 wk. These data demonstrate for the first time that STIM1 plays an essential role in normal cardiac function in the adult heart, which may be important for the regulation of ER and mitochondrial function.

Stromal interaction molecule 1 is a ubiquitously expressed type-I membrane protein, with its NH2-terminus including a canonical EF-hand Ca2+-binding domain located in the ER/SR lumen. The COOH-terminal region, which extends into the cytosol, contains a number of key regulatory domains that play a role in facilitating SOCE (5). In the currently accepted model for STIM1-mediated SOCE, a decrease in ER Ca2+ release results in dissociation of Ca2+ from STIM1, facilitating STIM1 oligomerization and subsequent accumulation of STIM1 in ER-plasma membrane junctions, where it interacts with Orai1 leading to Ca2+ entry (21, 33). While there are several variations of this model, the STIM1-Orai1 interaction is fundamental to the molecular mechanism of SOCE, and the knockdown of STIM1 attenuates SOCE (20, 22, 33, 38).

Although we and others have shown that both SOCE and STIM1/Orai1 exist in embryonic, neonatal, and adult cardiomyocytes (18, 30, 35, 42), it should be noted that the role of SOCE in adult cardiomyocytes remains controversial. Interestingly, while three separate groups have recently reported that STIM1 appears to be an essential component of the pathogenesis of cardiac hypertrophy (17, 22, 38), whether this is primarily through activation of SOCE remains to be determined. Importantly, there are also some store-independent functions of STIM1, including regulation of arachidonic acid-regulated Ca2+ (ARC) channels (36) and the maintenance of ER/SR structure (11).
Since STIM1 is highly conserved and appears to be ubiquitous in all eukaryotic cells (6), it seems likely that in addition to its pathophysiological role, STIM1 may also play an important physiological role in the heart; however, to date, remarkably little is known of the physiological role of STIM1 in the adult heart. Ubiquitous germ-line deletion of STIM1 is embryonically lethal (27); therefore, to determine the physiological role of STIM1 in the heart, we generated a cardiomyocyte-restricted STIM1 knockout ("STIM1-KO") mouse. We found that as early as 12 wk of age, there is evidence of ER stress and mitochondrial abnormalities in "STIM1-KO" hearts; between 20 and 36 wk of age, "STIM1-KO" mice exhibit a progressive decline in left ventricular (LV) function with the development of a dilated cardiomyopathy. This study demonstrates for the first time that STIM1 is essential for normal cardiomyocyte homeostasis potentially through maintenance of ER/SR and mitochondrial function.

MATERIALS AND METHODS

Materials. Unless otherwise stated, all reagents and chemicals were obtained from Fisher Scientific.

Cardiomyocyte-restricted STIM1 knockout mouse. All animal protocols were approved by the University of Alabama at Birmingham Institutional Animal Care and Use Committee and adhered to the National Institutes of Health’s Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, Revised 1996). All animals received standard chow and water on an ad libitum basis, and lighting was maintained on a 12-h:12-h light-dark cycle. Unless stated otherwise, the control animals used in this study refer to STIM1 flox/flox /MHC-cre mice (crSTIM1-KO). Data are reported for male mice only.

"STIM1-KO" mice were generated through breeding of homozygous STIM1 flox/flox mice, in which exon 2 of STIM1 was flanked by 2 lox P sites (28) with cardiomyocyte-specific α-MHC-cre; all mice were on a C57/Bl6 background. At 21 days of age, mice were weaned, tail-snipped, and ear-tagged, and genomic DNA was extracted from tail snips using a Qiagen Gentra Puregene kit. PCR was performed using genomic DNA obtained from tail snips, and as previously described, primer pairs were used to confirm STIM1 genotype (23) and expression of the α-MHC-cre transgene (26).

Adult cardiomyocytes were isolated from 20-wk control and "STIM1-KO" mice, as previously described (24, 25, 34).

Assessment of in vivo cardiac function. As previously described (41), mice were initially anesthetized using 5% isoflurane-95% O2 and restrained on a heated pad where chest hair was removed and a rectal temperature probe was inserted to ensure maintenance of body temperature. For measurements of cardiac function, the level of isoflurane was adjusted to 1.8%, and heart rate was continually assessed to ensure that cardiac function was not significantly depressed. Serial echocardiography was performed every 4 wk on adult male control and "STIM1-KO" mice between 12 and 36 wk of age using a VisualSonics Vevo 770 system in combination with a 30-MHz transducer.

Tissue analyses. For histological analyses, hearts were perfused with ice-cold PBS containing 25 mM KCl to clear blood and to arrest the heart. Hearts were then extracted, cut into transverse or longitudinal sections, and fixed for 24 h in 10% neutral buffered formalin and embedded in paraffin. Sections were cut at 5 μm and stained with hematoxylin-eosin, Masson trichrome, and picric acid Sirius red to assess cardiac morphology, cardiac fibrosis, and collagen deposition, respectively.

For transmission electron microscopy, LV tissue was isolated into longitudinal sections and placed in 0.1 mol/l cacodylate buffer containing 2% glutaraldehyde-paraformaldehyde and was heat-fixed for 30 min to cross link proteins and aldehydes. Lipid fixation was performed using 2% osmium tetroxide in 0.1 mol/l cacodylate buffer and 1% aqueous uranyl acetate and tissue embedded in Epon. Transmission electron microscopy was performed at the UAB high-resolution imaging facility, and longitudinal sections of tissue were assessed at the level of the ER/SR membrane, mitochondria, and contractile filaments.

To determine changes in protein levels, whole heart tissue or cardiomyocytes were initially homogenized/sonicated in lysis buffer containing 20 mM HEPES, 1.5 mM MgCl2, 20 mM KCl, 20% glycerol, 0.2 mM EGTA, 1% Triton X-100, 2 mM NaVO4, 10 mM NaF, and 2% protease inhibitor (pH 7.9). Protein was quantified using a Bio-Rad DC assay, and lysates were prepared using 6X sample buffer, consisting of 0.5 M Tris-HCl, 10% SDS, 30% glycerol, 0.2% β-mercaptoethanol, and 0.012% bromophenol blue, and boiled for 5 min. Protein (25 μg) was separated on 7.5% or 10% SDS-PAGE gels and subsequently transferred to polyvinylidene difluoride membranes. Wet polyvinylidene difluoride membranes were blocked for 1 h at room temperature with TBS-Tween 20 and 5% nonfat milk (except for STIM2, which was incubated with PBS-casem) and incubated overnight at 4°C with primary antibodies specific for STIM1 (1:1,000, Cell Signaling No. 4916), STIM2 (1:1,000, Cell Signaling No. 4917), Orai1 (1:1,000, ProSci No. 4281), mitofusin 2 (Mfn2; 1:1,500, Abcam), dynamin-related protein 1 (DRP-1, 1:1,000, Fisher Thermo Scientific), calsequestrin (1:5,000, Abcam No. 3516), and ER stress-specific antibodies (Cell Signaling No. 9956) for binding immunoglobulin protein/78 kDa glucose-regulated protein (BiP/GRP78; 1:1,000) and C/EBP homologous protein (CHOP; 1:1,000), inositol-requiring enzyme 1α (IRE1α; 1:500), and protein disulfide isomerase (PDI; 1:1,000), after which membranes were washed in TBS-Tween 20. Membranes were incubated with the appropriate horseradish peroxidase-conjugated secondary antibodies for 2 h at 4°C. Blots were exposed to enhanced chemiluminescence detection, and images were obtained using autoradiograph film. Expression was normalized to the loading control GAPDH (1:5,000, Santa Cruz Biotechnology), with β-actin (1:1,000, Abcam) in cardiomyocytes.

Statistical analyses. Statistical significance was calculated using a two-way repeated-measure ANOVA, followed by a Bonferroni post hoc test, as appropriate, using GraphPad Prism 4. P values of <0.05 were considered significant. Data are presented as means ± SE. All experiments comprise at least 3 to 4 mice per experimental manipulation. Densitometry of Western blots, quantification of cardiac fibrosis and collagen deposition, and quantification of mitochondrial size and number were performed using NIH ImageJ software.

RESULTS

To confirm deletion of STIM1 from cardiac tissue, we quantified STIM1 protein levels in both whole heart and isolated adult cardiomyocytes from 20-wk-old control and "STIM1-KO" mice. In whole heart tissue there was approximately a 20% reduction in total STIM1 protein expression in the "STIM1-KO" group compared with control (Fig. 1A); however, in cardiomyocytes isolated from "STIM1-KO" mice, STIM1 was virtually undetectable with a >90% reduction in its expression (Fig. 1B). These data clearly demonstrate the successful deletion of STIM1 in cardiomyocytes from "STIM1-KO" mice. The modest reduction in STIM1 in the whole heart reinforces the notion that STIM1 protein levels are relatively low in adult cardiomyocytes, relative to their levels in noncardiomyocyte cells such as smooth muscle cells, endothelial cells, and fibroblasts. Furthermore, to establish whether cardiogenic-specific deletion of STIM1 resulted in any compensa-
tory changes in other known SOCE proteins, we analyzed STIM2 and Orai1 levels in control and crSTIM1-KO cardiomyocytes and found no differences in expression between genotypes (Fig. 1C). In addition, analysis of STIM1 expression in skeletal muscle revealed no differences between control and crSTIM1-KO groups, confirming that the changes in STIM1 are cardiac-specific (data not shown). Since calsequestrin is responsible for buffering ER/SR Ca\textsuperscript{2+} levels, we examined levels of calsequestrin in cardiomyocytes isolated from 20-wk-old control and crSTIM1-KO mice. Control, \( n = 4 \); crSTIM1-KO, \( n = 4 \). Representative immunoblots (A–D, left) and mean densitometric data (A–D, right) from 4 individual experiments normalized to GAPDH (A) and \( \beta \)-actin (B–D) **\( P < 0.01 \) and *\( P < 0.05 \) vs. aged-matched control; Student’s t-test. ns, Not significant.

E: heat maps from unbiased gene expression array data were performed using the Illumina mouse WG-6 2.0 expression bead chip kit on hearts from crSTIM1-KO (\( n = 6 \)) and control (\( n = 6 \)) mice of 12 wk of age. Full gene name corresponds to *IGHV1S35_M12376_Ig_heavy_variable_1S35_13 and #IGHV1S120_AF025443_Ig_heavy_variable_1S120_8.

Fig. 1. Stromal interaction molecule 1 (STIM1) protein expression in whole heart tissue (A) and isolated cardiomyocytes (B) from adult male control and STIM1 knockout (crSTIM1-KO) mice. C: STIM2 and Orai1 expression in cardiomyocytes from adult male control and crSTIM1-KO mice. Control, \( n = 4 \); crSTIM1-KO, \( n = 4 \). Representative immunoblots (A–D, left) and mean densitometric data (A–D, right) from 4 individual experiments normalized to GAPDH (A) and \( \beta \)-actin (B–D) **\( P < 0.01 \) and *\( P < 0.05 \) vs. aged-matched control; Student’s t-test. ns, Not significant.

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Figure 2 shows that at 12 wk, there was a significant increase in both CHOP and PDI levels in the \(^{c}\text{STIM1-KO}\) group. At 20 wk, CHOP and PDI remained elevated, whereas BiP/GRP78 was significantly reduced. By 36 wk, CHOP and PDI were still increased, whereas BiP/GRP78 remained depressed and IRE1\(\alpha\) was significantly increased in \(^{c}\text{STIM1-KO}\) versus control. We also observed the same expression profiles for BiP, PDI, and CHOP in cardiomyocytes isolated from 20-wk control and \(^{c}\text{STIM1-KO}\) mice (Fig. 2C). Analyses of these same proteins in skeletal muscle show no differences between control and \(^{c}\text{STIM1-KO}\) groups, confirming that the changes in ER stress are cardiac-specific (data not shown).

There is growing appreciation of the importance of cross talk between ER/SR and mitochondria (8). Moreover, STIM1-deficient fibroblasts are reported to have altered mitochondrial function (14); therefore, we examined mitochondria morphology with transmission electron microscopy in 20-wk control and \(^{c}\text{STIM1-KO}\) mice. In Fig. 3, we show that at 20 wk of age, \(^{c}\text{STIM1-KO}\) mice exhibit pronounced ER/SR dilatation consistent with a disruption of the close ER-mitochondria association. In addition, mitochondria from \(^{c}\text{STIM1-KO}\) showed
heterogeneity in both size and number, resulting in increased numbers of smaller mitochondria, which were abnormally distributed throughout the tissue. We also observed that the mitochondria from crSTIM1-KO mice exhibited disruptions in cristae associated with mitochondrial fragmentation and increased focal vacuolization.

In light of the changes in mitochondrial morphology in crSTIM1-KO hearts, we quantified the levels the mitochondrial fission proteins FIS1 and DRP-1 and the mitochondrial fusion proteins Mfn1, Mfn2 and optic atrophy 1. We found that there was a significant increase in DRP-1 coupled with a significant reduction in Mfn2 levels in 20-wk crSTIM1-KO, with no change in Mfn1 expression (Fig. 3D). There were also no differences in FIS1 or optic atrophy 1 between groups (data not shown). These results are consistent with an increase in mitochondrial fission in crSTIM1-KO hearts and could be a contributing factor to the reduction in mitochondrial size and the increase in mitochondrial number we observe.

To determine whether the lack of STIM1 had any adverse effects on cardiac function, serial echocardiography was performed in crSTIM1-KO and control littermate mice every 4 wk, from 12 to 36 wk of age (Fig. 4). From 12 to 20 wk of age, there were no differences in cardiac function with respect to genotype; however, as shown in Fig. 4B, from 20 to 36 wk of age there was a progressive decline in ejection fraction in the crSTIM1-KO mice. Representative M-mode echo images at 12 and 36 wk as shown in Fig. 4A highlight the marked LV dilatation in the crSTIM1-KO mice at 36 wk, which is supported by the increase in LV volume (Fig. 4C) and decrease in ejection fraction (Fig. 4B). Although there was also a signifi-
cantly reduction in LV wall thickness between control and crSTIM1-KO, this difference was due to a significant increase in LV wall thickness in the controls between 20 and 36 wk of age. *P < 0.05 and **P < 0.001 vs. age-matched control (ANOVA/Bonferroni post hoc test). E: transverse whole heart images of hematoxylin and eosin (H&E) staining from 20- and 36-wk-old control and crSTIM1-KO, taken at 1.25×. Scale bar represents 2 mm. F: H&E, Masson trichrome, and picric acid Sirius red stained sections of hearts from 20- and 36-wk-old control and crSTIM1-KO. Images taken at 40×, and scale bar equals 50 μm. Control (20 wk, n = 6; and 36 wk, n = 7) crSTIM1-KO (20 wk, n = 14; and 36 wk, n = 8). G: Kaplan-Meier survival curve of crSTIM1-KO vs. age-matched control mice ($\chi^2$, P = 0.0001, n = 20 per genotype).

It has been reported that α-MHC-cre mice exhibit age-dependent decline in cardiac function (4); therefore, we performed serial echos on a cohort of α-MHC-cre and wild-type mice. Using two-way repeated-measure ANOVA, unlike the differences observed between crSTIM1-KO and control mice, we found by 36 wk of age there were no significant differences in ejection fraction, LV volume, or LV wall thickness between α-MHC-cre and wild-type mice (data not shown), confirming that induction of ER stress is
STIM1 was first identified in 1996 as a single-pass ER membrane protein with unknown function; however, in 2005, it was recognized as a required component of SOCE, and since then its role in regulating SOCE has been extensively examined, particularly in nonexcitable cells. It is also increasingly apparent that STIM1 has functions that are independent of SOCE, including regulation of ARC channels (36) and maintenance of ER/SR function (11). While there has been a growing body of evidence demonstrating that STIM1 is present in cardiomyocytes (19, 22, 37, 44), particularly as a mediator of cardiac hypertrophy (13, 17, 22, 38), the physiological role of STIM1 in the adult heart remains poorly understood. We have demonstrated for the first time that STIM1 plays an essential role in maintaining cardiomyocyte homeostasis. Specifically, we have shown that a >90% reduction in STIM1 expression in cardiomyocytes resulted in increased ER stress and changes in mitochondrial morphology consistent with increased mitochondrial fission that were associated with increased mortality, impaired LV function, and the development of a dilated cardiomyopathy.

The primary focus of STIM1 studies in the heart have been on the role of STIM1 in regulating hypertrophic signaling; however, the fact that STIM1 is evolutionarily highly conserved and is reported to be present in all eukaryotic cells (6) strongly suggests that it also plays an important homeostatic role. Earlier studies had reported that cardiomyocyte STIM1 protein levels decline from the embryonic to the adult heart, only to increase in response to pressure overload (17, 22). These observations raised the possibility that STIM1 might play an important role in early development of the heart and that like many stress-related proteins, it reemerges in the adult heart subjected to increased hemodynamic stress. However, if this were indeed the case, it would be anticipated that cSTIM1-KO would either be embryonically lethal or lead to severe phenotype in young animals. It is clear that this is not the case since cSTIM1-KO mice are born with the normal Mendelian frequency and survive into young adulthood with no overt phenotype. Indeed, the fact that there are no gross morphological or functional differences between cSTIM1-KO and control hearts up to 20 wk of age indicates that STIM1 is likely unnecessary for normal cardiac development and function. On the other hand, the rapid development of a dilated cardiomyopathy between 20 and 36 wk and significant mortality starting around 40 wk of age in the cSTIM1-KO group strongly suggests that STIM1 is required for maintaining normal cardiomyocyte homeostasis in the adult heart.

One indication of the potential importance of STIM1 in adult cardiomyocytes was evident in the gene array analyses where despite relatively minor differences between cSTIM1-KO and control groups, there were significant increases in brain natriuretic peptide and atrial natriuretic peptide, indicative of an early stress response. Furthermore, at 20 wk of age, even though functionally there were no differences between groups, there were increased inflammatory infiltrate and cardiac fibrosis in cSTIM1-KO hearts, indicative of additional cardiomyocyte stress and injury. While much of the focus on the role of STIM1 as a key mediator of SOCE and Ca\(^{2+}\) signaling, it has also been suggested that STIM1 may play a critical role in regulating ER/SR function to ensure appropriate folding and processing of proteins (37). This is supported by the fact that as early as 12 wk of age, there is a significant elevation in CHOP and PDI proteins in cSTIM1-KO hearts, which persists up to 36 wk of age. Interestingly, BiP/GRP78 protein is normal at 12 wk but is markedly reduced at 20 wk and remains depressed through 36 wk. The reduction in BiP/GRP78 protein levels is somewhat surprising since it is typically increased in response to acute ER stress and is responsible for binding to misfolded proteins to prevent aggregation, as well as the activation of CHOP. The lack of STIM1, however, likely represents a more chronic or sustained ER stress, and as such additional studies are required to understand the mechanism(s) contributing to the lower levels of BiP/GRP78. Nevertheless, these results are consistent with other reports indicating that STIM1 may mediate ER remodeling (11), as well as associate with several additional ER chaperone proteins such as stanniocalcin 2 (42) and the ER oxidoreductase ERP57 (32).

There is increasing evidence to show that there is a close association between ER/SR and mitochondria (8, 40) and that the progression of ER stress can lead to impaired mitochondrial function (2, 3). Interestingly, Henke et al. (14) reported that mouse embryonic fibroblasts lacking STIM1 exhibited abnormal mitochondrial function and morphology. These observations are consistent with our observations of altered mitochondrial morphology and disorganization in cSTIM1-KO hearts. Moreover, the changes in Mfn2 and DRP-1 seen here are indicative of a shift toward mitochondrial fission, which could account for the decrease in mitochondrial size and increased number of mitochondria observed in the cSTIM1-KO hearts. It should be noted that Mfn2 rather than Mfn1 has been shown to play a key role in mitochondrial ER/SR tethering (7) and may also regulate trafficking of STIM1 to the plasma membrane (35); consequently, the decrease in Mfn2 in the cSTIM1-KO hearts could be a contributing factor to the changes in mitochondrial morphology. While the mechanism underlying the decrease in Mfn2 in response to the loss of STIM1 remains to be determined, this observation underscores earlier reports indicating an interrelationship between Mfn2 and STIM1.

Cardiomyocytes are regularly exposed to an extracellular neurohormonal milieu, which includes the IP3-generating agonists, such as angiotensin II and endothelin, both of which are known to stimulate SOCE (29). It is possible, therefore, that in the setting of the cSTIM1-KO heart that the adverse effects we observe are due in part to the activation of IP3 receptor (IP3R) in the ER/SR in the absence of functional STIM1. If this indeed the case, then continued activation of IP3R could result in a prolonged reduction in ER/SR Ca\(^{2+}\) levels, normally sensed by STIM1, which may contribute to the observed induction of ER stress we observe since a reduction in ER/SR Ca\(^{2+}\) is known to induce ER stress (10, 12). It should also be noted that in addition to SOCE, STIM1 has been reported to regulate arachidonate-regulated Ca\(^{2+}\) (ARC) channels a store-independent Ca\(^{2+}\) entry pathway (36); consequently, a lack of STIM1 may also impair this Ca\(^{2+}\) entry pathway. However, since the function of ARC channels in the heart remains unclear, future
studies are needed to determine the impact of STIM1 deletion on ARC channel activity in isolated cardiomyocytes.

Our observation that STIM1 deficiency leads to dilated cardiomyopathy is supported by reports in the zebrafish, where inactivation of the Orai1 ortholog leads to heart failure and impaired mechanosignal transduction (39). In addition, Horton et al. (15) have shown that mice deficient in Orai1 develop a dilated cardiomyopathy that was associated with reduced survival without any evidence of cardiac hypertrophy. Humans lacking either STIM1 or Orai1 suffer from severe immunodeficiency and die at an early age from related complications; thus the effects on the heart would not be apparent. Consequently, the potential role of STIM1 in mediating cardiovascular disease in humans remains to be determined. However, it is worth noting that in a recent study, STIM1 polymorphisms were linked to increased risk for coronary artery dilatation (16). Although this study was performed in patients with Kawasaki disease, it raises the possibility that changes in STIM1 may also be associated with other cardiovascular pathologies and therefore needs to be further investigated. It is also of note that in mice, diabetes resulted in decreased STIM1 protein and ER Ca\textsuperscript{2+} levels in endothelial cells (9), and we have previously reported that hypertrophic signaling is impaired in cardiomyocytes from diabetic mice (24, 31). It is possible therefore that impaired STIM1 function could contribute to the increase risk of heart failure in diabetes. Interestingly, we have recently shown that increased modification of proteins by O-linked N-acetylglucosamine, which occurs in diabetes, also attenuated STIM1 function and SOCE (44).

The sustained expression of cre-recombinase in cardiomyocytes has been implicated in the development of impaired cardiac function. While this cannot be completely ruled out as a contributing factor in these studies, the fact that we did not see a decline in function in α-MHC-cre mice similar to STIM1-KO mice, would suggest this is not the case. In addition, the fact that there was no evidence of increased ER stress in 12 wk old in hearts from α-MHC-cre mice further supports the fact that these effects are specific for the lack of STIM1. However, this could be further confirmed using inducible, cardiomyocyte-specific STIM1-KO models, alternatively small interfering RNA approaches to chronically knockdown STIM1 as described by Hulot et al. (17) could be particularly valuable in this regard as they avoid the complications that can be associated with the different inducible KO models.

In conclusion, we have shown that the absence of STIM1 in cardiomyocytes is associated with ER stress as early as 12 wk of age as well as significant alterations in mitochondrial morphology consistent with an imbalance between mitochondrial fission and fusion and ultimately the development of a dilated cardiomyopathy and decreased life span. These findings are of particular importance, since the physiological role of STIM1 has been largely ignored in the heart. Clearly, much more needs to be done to fully understand the role of STIM1 in the heart, particularly including studies on cardiomyocyte function and Ca\textsuperscript{2+} handling at the single cell level; nevertheless, it is clear that this ER/SR Ca\textsuperscript{2+}-sensing protein is required for cardiomyocyte homeostasis and that may represent a novel link between ER/SR and mitochondrial function.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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