Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format. Questions? Contact Health Research Program staff at 717-783-2548.

1. **Grantee Institution:** Temple University – Of The Commonwealth System of Higher Education

2. **Reporting Period (start and end date of grant award period):** 1/1/2010-12/31/2013

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Germaine A Calicat, MLA

4. **Grant Contact Person's Telephone Number:** 215.204.7655

5. **Grant SAP Number:** 4100050909

6. **Project Number and Title of Research Project:** Project 10; PDMD: A Complex II-Regulated Form of Mitochondrial Dysfunction in Exhausted Cells

7. **Start and End Date of Research Project:** 12/1/2010-12/31/2011

8. **Name of Principal Investigator for the Research Project:** Madesh Muniswamy, Ph.D.

9. **Research Project Expenses**.

   9(A) Please provide the total amount of health research grant funds spent on this project for the entire duration of the grant, including indirect costs and any interest earned that was spent:

   $ 46,248.28

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name, First Name	Position Title	% of Effort on Project	Cost
Daswani	Graduate Student	100	$27,845

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name, First Name	Position Title	% of Effort on Project

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment. None

Type of Scientific Equipment	Value Derived	Cost

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes_____ No____x_____

If yes, please indicate the source and amount of other funds:

11. Leveraging of Additional Funds

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes_____ No_____X_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds awarded:
	☐NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)		$	$
	☐NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)		$	$
	☐NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)		$	$

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes_____ No____x_____

If yes, please describe your plans:

12. Future of Research Project. What are the future plans for this research project?

None

13. New Investigator Training and Development. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes_____ No_____x_____

If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male				
Female				
Unknown				
Total				

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic				
Unknown				
Total				

	Undergraduate	Masters	Pre-doc	Post-doc
White				
Black				
Asian				
Other				
Unknown				
Total				

14. Recruitment of Out-of–State Researchers. Did you bring researchers into Pennsylvania to carry out this research project?

Yes_____ No___x_____

If yes, please list the name and degree of each researcher and his/her previous affiliation:

15. Impact on Research Capacity and Quality. Did the health research project enhance the quality and/or capacity of research at your institution?

Yes____ _____ No____X_____

If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

16. Collaboration, business and community involvement.

16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes_____ No____x_____

If yes, please describe the collaborations:

16(B) Did the research project result in commercial development of any research products?

Yes_____ No___x_____

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes_____ No_____x_____

If yes, please describe involvement with community groups that resulted from the research project:

17. Progress in Achieving Research Goals, Objectives and Aims.
List the project goals, objectives and specific aims (as contained in the grant agreement). Summarize the progress made in achieving these goals, objectives and aims <u>for the period that the project was funded (i.e., from project start date through end date).</u> Indicate whether

or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

Specific Aim 1: Characterize the role of PDMD in mitochondrial physiology

a. Role of individual ETC complexes in $\Delta\Psi_m$ maintenance and activation of PDMD.
b. Role of PDMD in hypoxic condition.
c. Role of PDMD in cellular and mitochondrial bioenergetics and respiration rate.
d. Is PDMD evolutionarily conserved throughout the eukaryotic system?

Progress made on aim 1: The proposed Specific Aim 1 was successfully completed.
A). Role of individual ETC complexes in $\Delta\Psi_m$ maintenance and activation of PDMD.
The complex II pathway preserves the membrane potential in the absence of complex I activity.
As Succinate afforded protection from $\Delta\Psi_m$ loss during pseudo-hypoxia, we next chose to investigate the discrete role of complex II in $\Delta\Psi_m$ maintenance. To assess the contribution of individual mitochondrial complexes to the $\Delta\Psi_m$, permeabilized rat pulmonary microvascular endothelial cells (RPMVECs) were supplemented with complex-specific mitochondrial substrates either alone or in combination with mitochondrial complex and F_0F_1-ATPase inhibitors. Following permeabilization, cells exhibit $\Delta\Psi_m$ loss due to lack of available substrates as the cytoplasmic contents diffuse and dilute into the experimental buffer (data not shown). Surprisingly, the complex II substrate succinate (Succ), but not the complex I substrates malate

and glutamate (Mal/Glut), effectively maintained $\Delta\Psi_m$ in response to permeabilization (Fig. 1A). Succ also prevented $\Delta\Psi_m$ loss even in the presence of the F_0F_1-ATPase inhibitor Oligomycin, which expectedly blocked the protective effect of ATP on $\Delta\Psi_m$ maintenance (Fig. 1B). Further, the effect of Succ on $\Delta\Psi_m$ maintenance was not affected by the complex I inhibitor Rot (Fig. 1C), excluding the possibility that Succ initiates proton pumping via the reverse flow of electrons through complex I, and was completely abolished by the non-metabolizable complex II competitive inhibitor malonate (Mao) (Fig. 1D). However, Succ did not offer protection from complex III-mediated $\Delta\Psi_m$ loss in the presence of the inhibitors myxothiazol and antimycin A (Fig. 1E and F). In particular, antimycin A induced immediate $\Delta\Psi_m$ loss even in the combined presence of Succinate and ATP, which hyperpolarizes the membrane, suggesting that complex II requires the proton pumping capability of mitochondrial complex III in order to establish $\Delta\Psi_m$. This initial observation raises the possibility that complex II pathway may be sufficient to establish $\Delta\Psi_m$ in the absence of complex I.

B). <u>Role of PDMD in hypoxic condition:</u>To assess establishment of $\Delta\Psi_m$ during hypoxic conditions, we chose to utilize freshly isolated murine cardiomyocytes as these cells are a primary target of hypoxic damage in humans during myocardial infarction. Chemical inhibition of complexes I and IV (Rot/Oligo) induced rapid $\Delta\Psi_m$ loss in cardiomyocytes that was similar to other cell types that could be reversed by Succ supplementation (Fig. 2A). To directly assess the relationship between $\Delta\Psi_m$ loss and hypoxia, $\Delta\Psi_m$ was assessed following cell permeabilization in hypoxic experimental buffer. Marked $\Delta\Psi_m$ loss was observed when cells were permeabilized in this buffer (Fig. 2B), the hypoxic status of which was verified by probing for HIF-1α stabilization after 5 hr (Fig. 2C).

<u>D) Is PDMD evolutionarily conserved throughout the eukaryotic system?</u> To further characterize the role of complex II in the $\Delta\Psi_m$, we next assessed whether reintroduction of the complex II substrate Succ could restore $\Delta\Psi_m$ after complete dissipation of the proton gradient ($\Delta\Psi_m$ loss). Following permeabilization-induced $\Delta\Psi_m$ loss, cells were supplemented with either a combination of complex I substrates (Mal/Glut or Mal/Pyr) or Succ. In contrast to Mal/Glut or Mal/Pyr, addition of Succ resulted in dramatic $\Delta\Psi_m$ restoration (Fig. 3A). Because complex I substrates were ineffectual at maintaining $\Delta\Psi_m$, we developed a rapid chemical means to further test the efficacy of complex II in mitochondrial function and mimic hypoxia in normoxic cells. Cells were supplemented with Mal/Glut and then $\Delta\Psi_m$ loss was induced by a combination of Rot/Oligo, completely eliminating the contribution of complex I (via secondary ATP production) as well as intermembrane space proton loss through F_0F_1-ATPase. Cells were then treated with the complex II substrate Succ and extramitochondrial ATP alone or in combination. This approach (Succ versus ATP) allowed us to distinguish between proton gradient maintenance by Succ or glycolytic ATP, and to directly monitor $\Delta\Psi_m$ recovery. The chemical combination of Rot/Oligo triggered rapid $\Delta\Psi_m$ loss, which could be reversed by supplementation with Succ in several cell types that we have examined (mouse, rat, and human pulmonary microvascular endothelial cells (MPMVEC, RPMVEC, and HPMVEC, respectively), C2C12 myoblast, and S2 drosophila cells) (Fig. 3B, C, F). Complex III inhibition resulted in $\Delta\Psi_m$ loss that could not be rescued by the proton-pumping capabilities of complex I alone. While ATP in combination with Succ produced an additive effect on $\Delta\Psi_m$, ATP alone did not reverse $\Delta\Psi_m$ loss (Fig. 3B). The fatty acid β-oxidation end-product acetyl CoA is a major fuel in some tissues, and ultimately enters the citric acid cycle where it will increase all TCA cycle metabolites, including Succ and

Mal. However, Rot/Oligo-induced $\Delta\Psi_m$ loss was not affected by acetyl CoA supplementation (Fig. 3C). Addition of the citric acid cycle metabolite α-ketoglutarate also did not restore $\Delta\Psi_m$, despite being a precursor of Succ in the TCA cycle (Fig. 3C). In addition to Rot/Oligo, $\Delta\Psi_m$ loss also occurred following addition of the complex II competitive inhibitor Malonate (Mao). Subsequent addition of the complex I substrates Mal/Pyr and their essential cofactor NAD+ did not restore $\Delta\Psi_m$ (Fig. 3D), indicating a rapid effect of Succ in the establishment of the $\Delta\Psi_m$. Although our data demonstrate the restoration of $\Delta\Psi_m$ by Succ following $\Delta\Psi_m$ loss, there is a possibility that restoration of $\Delta\Psi_m$ by Succ is actually due to the secondary generation of NADH from mitochondria. To address this issue, we simultaneously measured both $\Delta\Psi_m$ and NADH generation in permeabilized cells. Inhibition of complex I by Rot rapidly increased cellular NADH levels but did not affect $\Delta\Psi_m$ as detected by TMRE fluorescence. However, subsequent addition of Succ rapidly increased $\Delta\Psi_m$ (as indicated by a decrease in TMRE as more dye sequesters into the mitochondria) without an appreciable effect on NADH generation (Fig. 3E). Conversely, Mal/Glut did not increase $\Delta\Psi_m$ when complex II was inhibited by Mao despite a robust increase in cellular NADH levels. Because Succ-mediated electron transfer relies on complex III (Fig. 2E and F), we next assessed whether bypass of complex II/III by delivery of the complex IV substrates tetramethyl-*p*-phenylenediamine (TMPD) and ascorbate (Asc) could preserve $\Delta\Psi_m$ in response to a combined complex I/F_0F_1-ATPase inhibition (Rot/Oligo). TMPD/Asc supplementation only partially restored $\Delta\Psi_m$ (Fig. 3F). However, unlike the dose-dependent response seen with Succ, addition of a higher concentration of TMPD/Asc was toxic to mitochondria and $\Delta\Psi_m$ maintenance.

Specific Aim 2: Does the re-establishment of the proton electrochemical gradient restore mitochondrial function?

a. Does cyclophilin D inhibition re-establish the $\Delta\Psi_m$following PDMD?
b. Is PDMD a Bcl-2 family protein-associated mechanism?
c. Does supplementation of the citric acid cycle metabolite succinate restore $\Delta\Psi_m$ and cell survival in intact cells?

Progress made on aim 2: A). Does cyclophilin D inhibition re-establish the $\Delta\Psi_m$following PDMD?

Rot/Oligo-induced mitochondrial depolarization was not prevented by the PTP blocker cyclosporine A (CsA), but subsequent addition of the complex II substrate succinate (Succ) substantially restored $\Delta\Psi_m$. Pretreatment with CsA also did not prevent Rot/Oligo-induced $\Delta\Psi_m$ loss, although it significantly blunted Ca^{2+}-dependent mitochondrial depolarization via the PTP open even in the presence of Succ, indicating that Succ cannot prevent opening of the PTP. In parallel, Succ supplementation did not prevent $\Delta\Psi_m$ loss triggered by the mitochondrial swelling-inducing agent alamethicin (Fig. 4A-C).

B). Is PDMD a Bcl-2 family protein-associated mechanism?

Next, it was of interest to determine whether Rot/Oligo-induced $\Delta\Psi_m$ loss is similar to mitochondrial depolarization caused by pro-apoptotic Bcl-2 family proteins. A logical assumption would be that the mitochondrial proton gradient can be maintained if complex III and complex IV are intact and cytochrome c is present to enable electron transfer from complex III to IV. During apoptosis, cytochrome c is released from the intermembrane space via a Bax/Bak-dependent mechanism that precedes $\Delta\Psi_m$ loss. Rot/Oligo-induced $\Delta\Psi_m$ loss is a Bcl-2

independent phenomenon. $\Delta\Psi_m$ loss by the pro-apoptotic protein tBid (caspase-8-cleaved), an activator of Bax and Bak, is not rescued by Succ (Fig. 4D). This observation suggests that cytochrome c is essential for normal $\Delta\Psi_m$ maintenance initiated by complex II. Similar to other cell types, WT and *bax*$^{-/-}$*bak*$^{-/-}$ double KO fibroblasts underwent Succ-reversible $\Delta\Psi_m$ loss even in the presence of CsA (Fig. 4E and F). Our data suggests that Rot/Oligo-induced mitochondrial depolarization is neither an apoptotic (Bcl-2-mediated) nor a necrotic (PTP opening) phenomenon, and thus should not exhibit mitochondrial cytochrome c release. Unlike tBid (100 nM), Rot/Oligo-induced depolarization did not trigger cytochrome c release after 20 min exposure (Fig. 4G). These results demonstrate that Rot/Oligo induces $\Delta\Psi_m$ loss by mechanisms not directly dependent upon Bcl-2 or PTP opening and suggests that mitochondrial membrane potential may be manipulated during hypoxia via the availability of specific metabolic substrates.

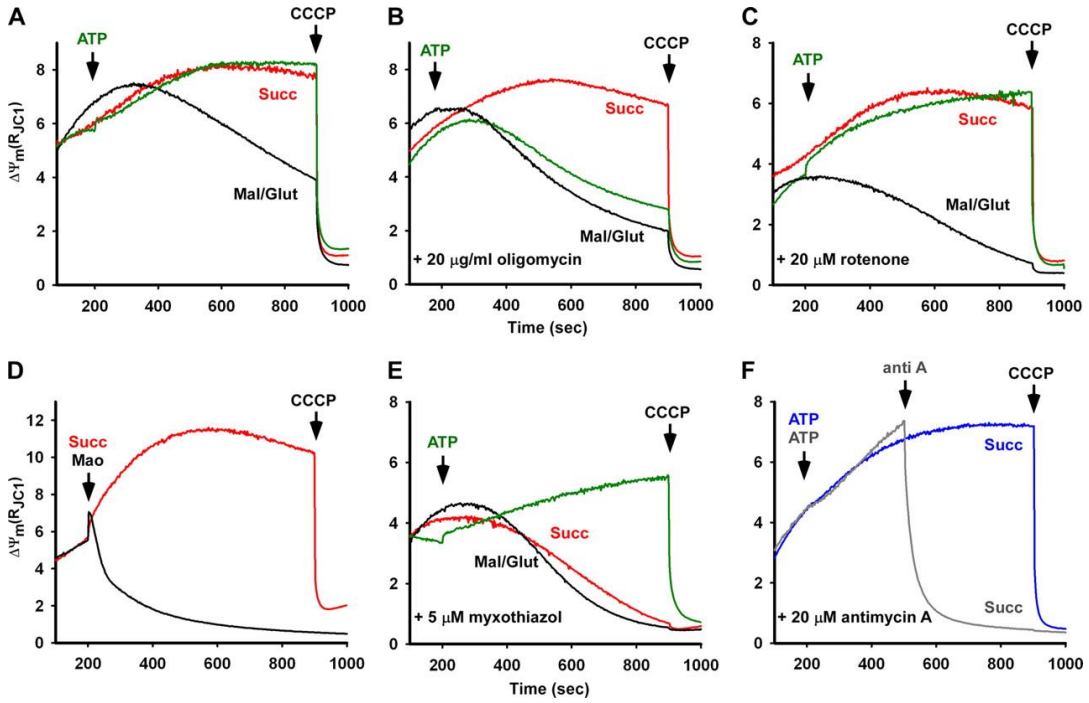

Fig. 1. Mitochondrial complex II establishes the membrane potential in permeabilized cells. Succinate (Succ; 2 mM) but not malate/glutamate (Mal/Glut; 1 mM/1 mM) maintained $\Delta\Psi_m$ in digitonin-permeabilized RPMVECs under (A) control cells and (B) in the presence of the F_0F_1-ATPase inhibitor oligomycin (Oligo; 20 μg/ml). ATP (100 μM) was effective only in the absence of oligomycin. (C) Complex I inhibition by rotenone (Rot; 20 μM) does not affect Succ-mediated $\Delta\Psi_m$ maintenance but (D) the complex II inhibitor malonate (Mao; 2 μM) abolished Succ-dependent $\Delta\Psi_m$. Succ afforded no protection from complex III inhibition by (E) myxothiazol (5 μM) or (F) antimycin A (20 μM). Arrows indicate the addition of different molecules at various intervals. The mitochondrial uncoupler CCCP was added at the indicated time to dissipate the membrane potential.

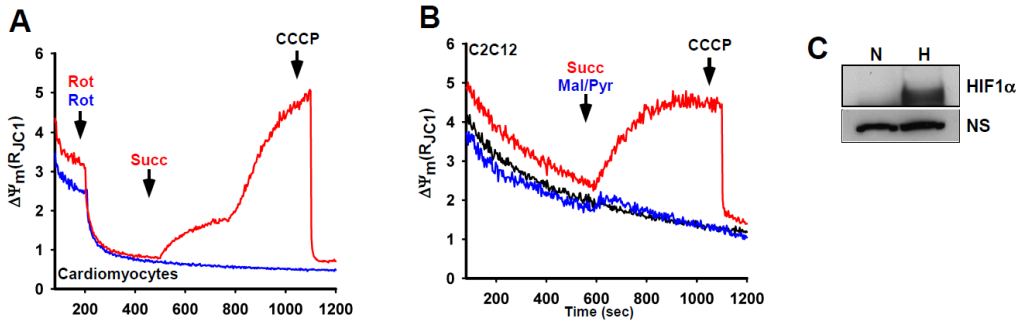

Fig. 2. Preservation of mitochondrial membrane potential by Succ-mediated metabolism during hypoxia. Both (A) chemical- and (B) hypoxic-induced $\Delta\Psi_m$ loss is reversed by Succ but not Mal/Pyr in freshly isolated permeabilized, mature murine cardiomyocytes and C2C12 cells, respectively. (C) Hypoxic status was confirmed by HIF-1α stabilization in C2C12 cells following 5 hr of hypoxia (NS; non specific).

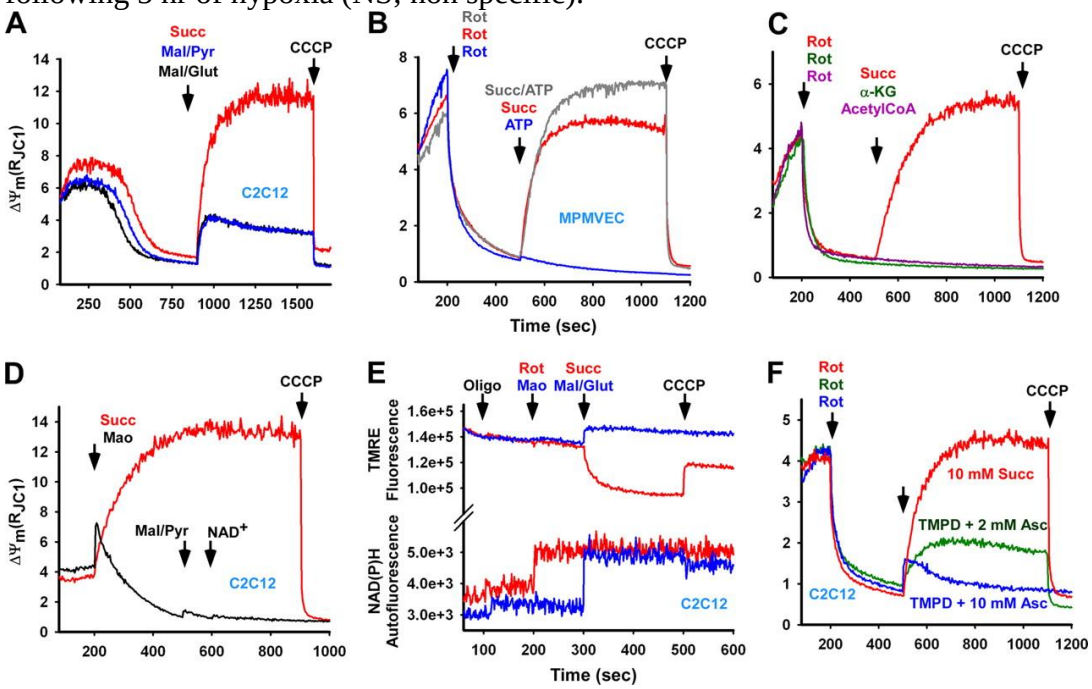

Fig. 3. Complex II substrate restores $\Delta\Psi_m$ during complex I and F_0F_1-ATPase inhibition. (A) Differential effects of Complex II (Succ; 10 mM) and Complex I (Mal/Glut; 5 mM/5 mM, Mal/Pyr; 5 mM/ 5 mM) substrates on reestablishment of $\Delta\Psi_m$ in murine myoblast (C2C12) cell line. (B) $\Delta\Psi_m$ depolarization caused by complex I and F_0F_1-ATPase inhibition (Rot/Oligo; 20 μM/ 20 μg/ml) could be reversed by Succ (10 mM) in MPMVECs. (C) The citric acid entry substrate Acetyl CoA (AcetylCoA; 5 mM), and the Succ precursor α-ketoglutarate did not restore $\Delta\Psi_m$ in HPMVECs. (D) Mao-induced $\Delta\Psi_m$ depolarization was not reversed by combination of the complex I substrates Mal/Pyr (5 mM/5 mM) and the cofactor NAD^+ (100 μM). (E) An increase in NADH production in response to Mal/Glut (5 mM/5 mM) did not restore $\Delta\Psi_m$ in response to complex II inhibition by Mao (2 mM). (F) The complex IV substrates TMPD/Asc do not effectively reestablish $\Delta\Psi_m$ following Ros/Oligo-induced $\Delta\Psi_m$ loss. Oligo was added at 80 sec in B, C, D, and F. Arrows indicate the addition of different molecules at various intervals.

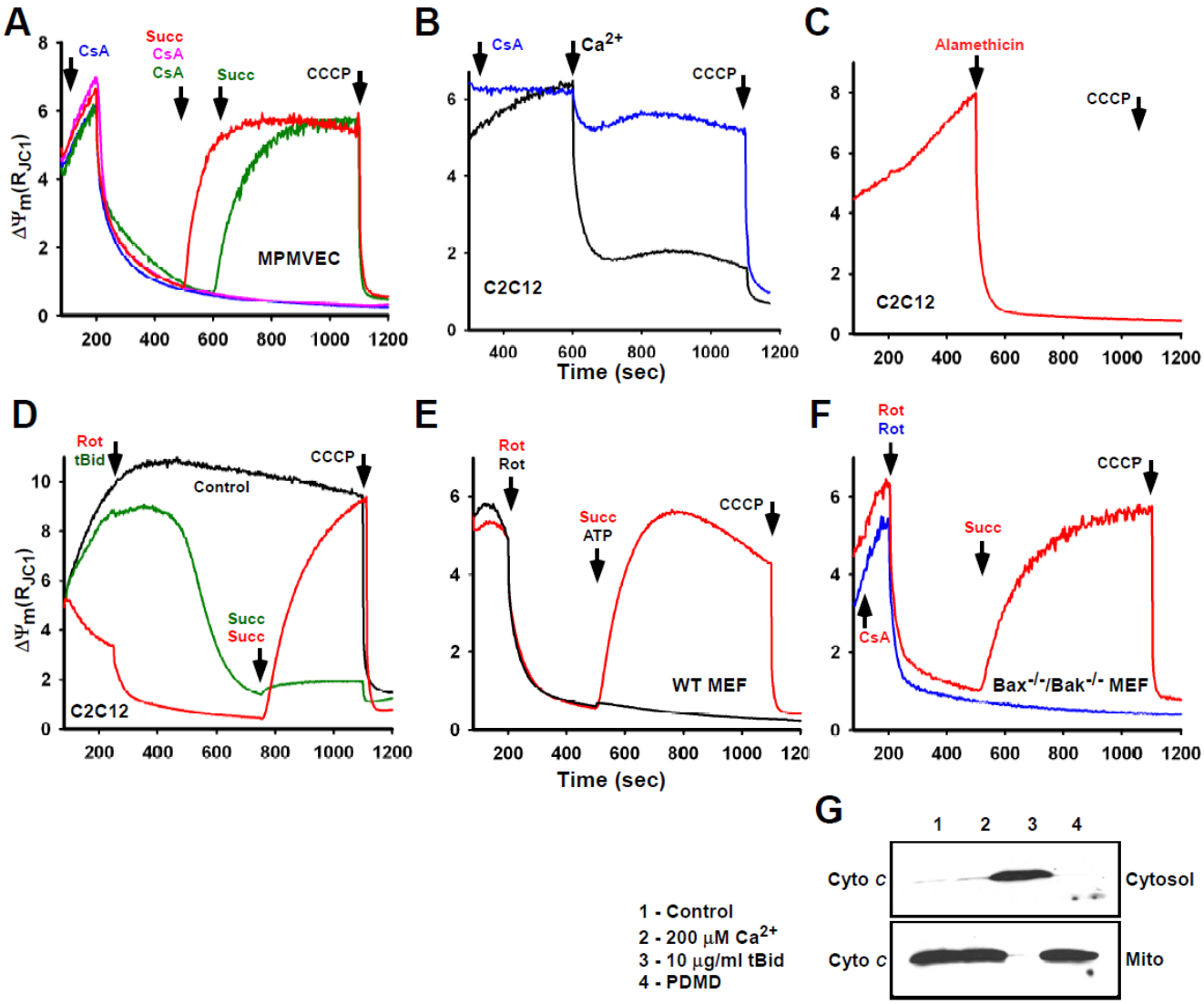

Fig. 4. Complex II-regulated PDMD is independent of PTP and Bcl-2 apoptotic OMM permeabilization. (A) Complex I/ F_0F_1-ATPase inhibition-induced PDMD was reversed by Succ but not the PTP blocker CsA (5 μM). (B) In contrast, Ca^{2+}-induced mitochondrial depolarization is blocked by CsA but not Succ. (C) Succ-dependent $\Delta\Psi_m$ maintenance is abolished by mitochondrial swelling triggered by the microbial antibiotic alamethacin. Note: (B) and (C) were performed in the presence of Succ. (D) Moreover, Bcl-2 family protein tBid-induced $\Delta\Psi_m$ loss is not prevented by Succ. (E) Wild type and (F) $bax^{-/-}bak^{-/-}$ double-deficient murine embryonic fibroblasts subjected to Oligo/Rot-induced PDMD. Double-deficient cells did not prevent Oligo/Rot-induced PDMD, but Succ restored $\Delta\Psi_m$. (G). Oligo/Rot-induced PDMD did not trigger cytochrome c release from the mitochondria.

Specific Aim 3: Is PDMD a primary mechanism of cell injury?

a. Is PDMD conserved in primary cardiomyocytes?

b. Is the nuclear encoded mitochondrial complex II a crucial regulator of PDMD.

c. Distinguishing the roles of complex I and complex II in PDMD.

Progress made on aim 3:

a. Is PDMD conserved in primary cardiomyocytes?

To assess establishment of $\Delta\Psi_m$ during hypoxic conditions, we chose to utilize freshly isolated murine cardiomyocytes as these cells are a primary target of hypoxic damage in humans during myocardial infarction. Chemical inhibition of complexes I and IV (Rot/Oligo) induced rapid $\Delta\Psi_m$ loss in cardiomyocytes that was similar to other cell types that could be reversed by Succ supplementation (Fig. 5A). To directly assess the relationship between $\Delta\Psi_m$ loss and hypoxia, $\Delta\Psi_m$ was assessed following cell permeabilization in hypoxic experimental buffer. Marked $\Delta\Psi_m$ loss was observed when cells were permeabilized in this buffer (Fig. 5B), the hypoxic status of which was verified by probing for HIF-1α stabilization after 5 hr (Fig. 5C). Succ but not Mal/Pyr supplementation restored $\Delta\Psi_m$ in response to hypoxia (Fig. 5B).

b. Is the nuclear encoded mitochondrial complex II a crucial regulator of PDMD.

Mitochondria are the only organelles outside the nucleus that contain DNA (mtDNA) and the machinery for synthesizing RNA and proteins. Mitochondrial complexes I, III and IV and F_0F_1-ATPase are multi-subunit protein complexes derived from both the nuclear and mitochondrial genome. In contrast, complex II polypeptides are derived solely from nuclear DNA. Many maternally inherited mitochondrial diseases arise from mutations in mitochondrial complexes I, III and IV and F_0F_1-ATPase, including Kearns-Sayre syndrome (KSS) – which results from a 1.9-kb mtDNA deletion spanning both complex I (ND1, ND2, ND4, NDL, and ND6 gene products) and F_0F_1-ATPase (ATP6 and ATP8 genes) and Leber hereditary optic neuropathy (LHON), which affects subunit 4 of complex I. Primary fibroblasts from these patients therefore constitute an ideal model to identify the unique role of complex II in $\Delta\Psi_m$ maintenance. Cells derived from LHON (Fig. 6B) and KSS (Fig. 6C) patients exhibited a similar $\Delta\Psi_m$ response to Rot/Oligo as control fibroblasts (Fig. 5A) that was reversed by Succ. Intact CF9, LHON, and KSS cells also exhibited a similar phenotype with a predominance of filamentous mitochondria (Fig. 6D, E, F) and displayed a similar $\Delta\Psi_m$ that was established principally by mitochondrial complex II (Fig. 6G – J). Because Mao addition resulted in $\Delta\Psi_m$ loss in all three cell types, this evidence also suggests that LHON and KSS cells do not utilize a compensatory mechanism to generate basal $\Delta\Psi_m$, but rather, all three cells establish $\Delta\Psi_m$ predominately through a mitochondrial complex II dependent pathway. An intact membrane potential generated by complex II also allows for LHON and KSS cells to sequester cytosolic calcium in an identical pattern to that of CF9 control cells (Fig. 6K), indicating that $\Delta\Psi_m$-dependent mitochondrial function is largely intact and dependent upon establishment of the IMM proton gradient by complex II. However, despite the ability to generate an intact membrane potential, complex I deficiencies lead to a compensatory glycolytic shift and a decrease in ATP generation. Accordingly, LHON cells consumed significantly less oxygen than both KSS and CF9 cells (Fig. 6L). The fact that the LHON phenotype exhibited less oxygen utilization than KSS cells was surprising considering only complex I was affected, and led us to consider the possibility that establishment of the $\Delta\Psi_m$ would be possible by diverting cells into succinate-driven metabolism during reduced oxygen availability.

c. Distinguishing the roles of complex I and complex II in PDMD.

While it is possible that the nominal effect of complex I substrates on $\Delta\Psi_m$ maintenance during hypoxia could be due the inactivation or enhancement of specific mitochondrial complexes, no differences in the activity of either complex I or complex II were noted (Fig. 5D). Similar to the permeabilized cell model, intact C2C12 and freshly isolated cardiomyocytes subjected to hypoxic conditions (Fig. 5E) exhibited a significant reduction in $\Delta\Psi_m$ that was effectively restored by supplementation with complex II but not complex I substrates in the hypoxic medium (Fig. 5E, F, G, H). Active mitochondrial complexes support the generation of an electrochemical gradient across the IMM, which is vital for the efficient production of ATP (33). We therefore analyzed the role of complex II-dependent $\Delta\Psi_m$ restoration in the maintenance of ATP levels under hypoxia. While less than that during normoxia, Succ supplementation resulted in significant ATP level maintenance, consistent with the restoration of $\Delta\Psi_m$ by Succ in response to hypoxia (Fig. 5I).

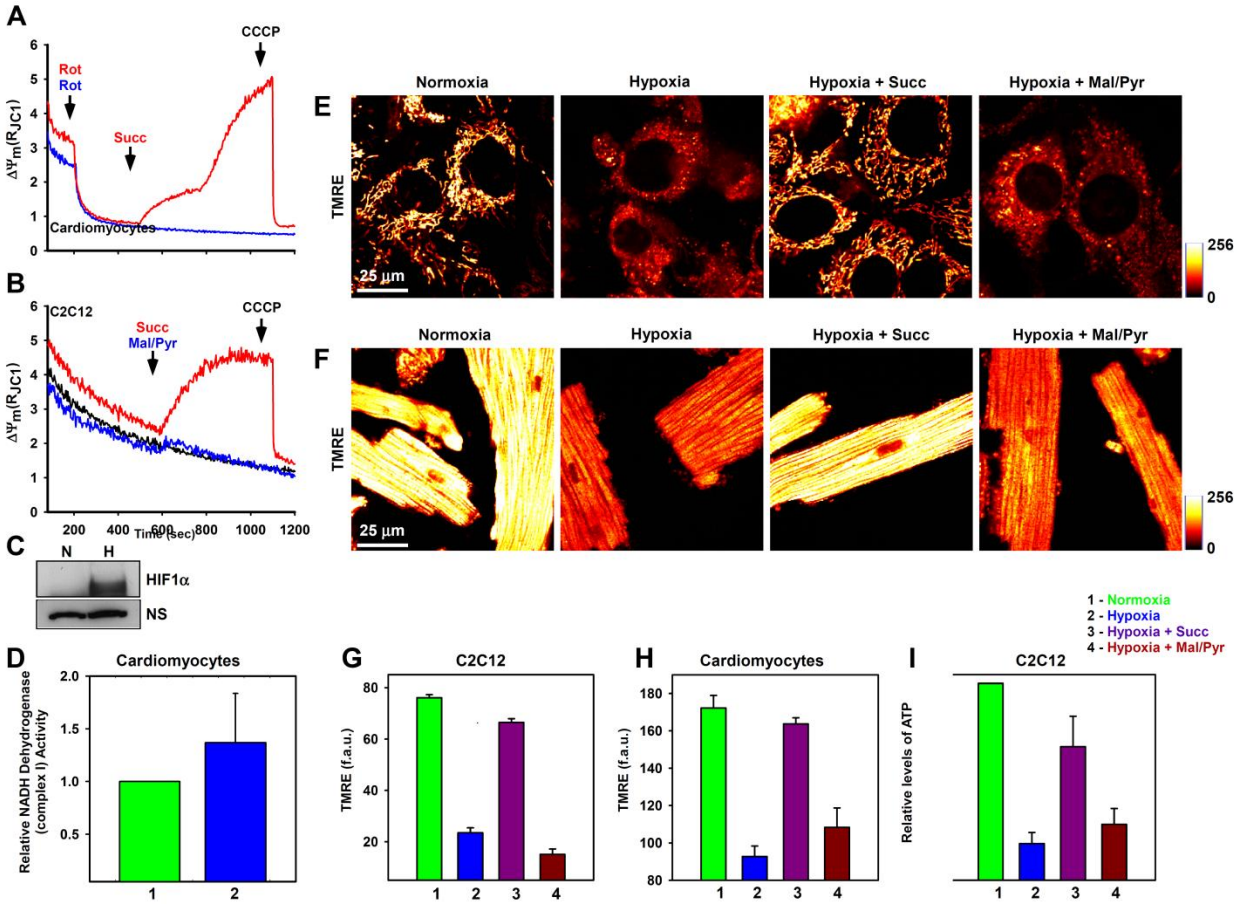

Fig. 5. Preservation of mitochondrial function and energy metabolism by Succ-mediated metabolism during hypoxia. Both (A) chemical- and (B) hypoxic-induced $\Delta\Psi_m$ loss is reversed by Succ but not Mal/Pyr in freshly isolated permeabilized, mature murine cardiomyocytes and C2C12 cells, respectively. (C) Hypoxic status was confirmed by HIF-1α stabilization in C2C12 cells following 5 hr of hypoxia (NS;

non specific). (D) Primary adult cardiomyocyte mitochondrial complex I and complex II activity were unaltered during hypoxia. (E) Intact C2C12 or (F) freshly isolated adult cardiomyocytes were subjected to hypoxic conditions for 5 hr and cells were loaded with the $\Delta\Psi_m$ indicator TMRE. Normoxic and Succ-supplemented hypoxic cells retained $\Delta\Psi_m$. In contrast, hypoxia alone or supplemented with Mal/Pyr caused mitochondrial depolarization. (F) and (G) Average fluorescence intensity of mitochondrial TMRE retention as described in (F) and (G) following normoxic and hypoxic conditions. (H) ATP level maintenance by Succ supplementation in response to hypoxia. All values in D, G, H, and I indicative mean + s.e.m. (n=3).

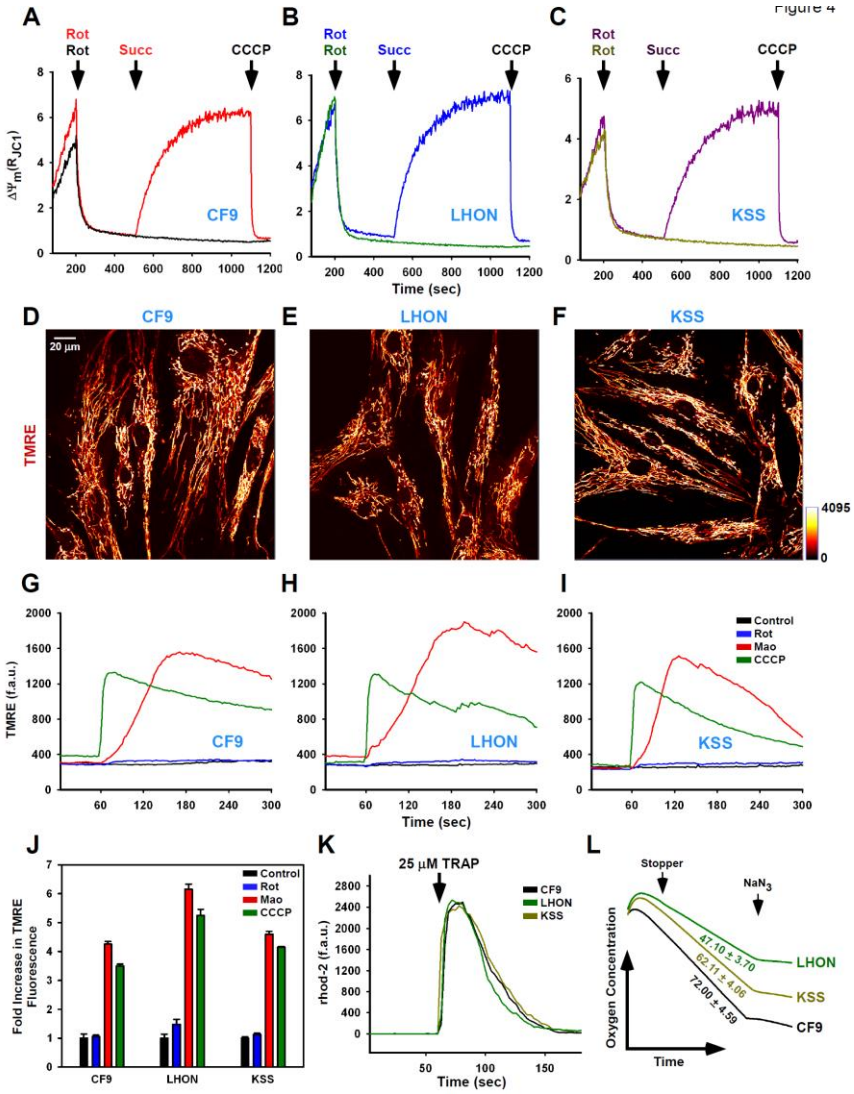

Fig. 6 Establishment of $\Delta\Psi_m$ in human fibroblasts from patients with mitochondrial disorders. (A) Control human fibroblasts (CF9) exhibit $\Delta\Psi_m$ loss in response to Rot/Oligo that is reversed by Succ (10 mM). Similar responses were observed in human clinical samples from (B) LHON and (C) KSS patients. (D) CF9 control and (E) LHON and (F) KSS patient samples display a similar mitochondrial morphology as determined by the cationic dye TMRE. Mitochondrial depolarization, as detected by the presence of TMRE in the nucleus, occurs in response to Mao (5 mM) but not Rot (20 µM) in (G) CF9, (H) LHON, and (I) KSS cells. CCCP was used as a positive control. (J) Quantitation of nuclear TMRE fluorescence increase in response to Rot, Mao, and CCCP in CF9, LHON, and KSS cells. Values indicate mean ± s.e.m. (n=3). (K) Mitochondrial Ca^{2+} uptake is similar in all cell types in response to the protease-activated receptor agonist peptide TRAP (25 µM). (L) Whole cell mitochondrial oxygen consumption of CF9, LHON, and KSS cells ($8x10^6$). Stopper indicates when the oxygen consumption chamber was sealed. Sodium Azide (NaN_3) was used to inhibit cellular oxygen consumption at complex IV as a positive control. Values indicate oxygen consumption as nmoles oxygen/min/$8x10^6$ cells (mean ± s.e.m.; n=3).

18. Extent of Clinical Activities Initiated and Completed. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
____x__No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
___x___No

If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

_____Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

_____Number of subjects originally targeted to be included in the study
_____Number of subjects enrolled in the study

Note: Studies that fall dramatically short on recruitment are encouraged to provide the details of their recruitment efforts in Item 17, Progress in Achieving Research Goals, Objectives and Aims. For example, the number of eligible subjects approached, the number that refused to participate and the reasons for refusal. Without this information it is difficult to discern whether eligibility criteria were too restrictive or the study simply did not appeal to subjects.

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics
_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_____White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

19. Human Embryonic Stem Cell Research. Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____Yes
____x_ No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?
_____Yes
__x___No

19(C) Please describe how this project involved human embryonic stem cells:

20. Articles Submitted to Peer-Reviewed Publications.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication or paper submitted for publication, listed in the table, in a PDF version 5.0.5 (or greater) format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, and an abbreviated title of the publication. For example, if you submit two publications for Smith (PI for Project 01), one

publication for Zhang (PI for Project 03), and one publication for Bates (PI for Project 04), the filenames would be:

Project 01 – Smith – Publication 1 – Cognition and MRI
Project 01 – Smith – Publication 2 – Cognition and MRI
Project 03 – Zhang – Publication 1 – Lung Cancer
Project 03 – Zhang – Publication 2 – Lung Cancer

If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
1.				☐Submitted ☐Accepted ☐Published
2.				☐Submitted ☐Accepted ☐Published
3.				☐Submitted ☐Accepted ☐Published

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes_____ No____x_____

If yes, please describe your plans:

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project. Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes_____ No___x_____

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____
If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No____

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes___ No____

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes_____ No____x_____

If yes, please describe your plans:

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages. *For Nonformula grants only – include information for only those key investigators whose biosketches were not included in the original grant application.*

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR** PAGES.

NAME	POSITION TITLE
Madesh Muniswamy	Assistant Professor of Biochemistry and Center for Translational Medicine
eRA COMMONS USER NAME MUNISWAMY	

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of Madras	B.S.	1991	Biochemistry
University of Madras	M.S.	1987	Biochemistry
The Wellcome Trust Research Laboratory	Ph.D.	1990	Bio-Medical Sciences

A. Personal Statement

My laboratory identified major mitochondrial Ca^{2+} signaling components and that uniguely places us in a privileged position to investigate the proposed hypothesis. Our very recent discoveries demonstrated that a mitochondrial resident transmembrane protein Mitochondrial Ca^{2+} Uniporter Regulator 1 (**MCUR1**) is essential for Mitochondrial Ca^{2+} Uniporter (MCU)-mediated mitochondrial Ca^{2+} uptake (**Nature Cell Biology** see #12; Highlights in Nature Reviews Molecular Cell Biology). We also identified the molecular component (Mitochondrial Ca^{2+} \Uptake 1; **MICU1**) that controls mitochondrial Ca^{2+} uptake "set point" a concept known over thirty years (**Cell** see #11). I have a broad background in mitochondrial physiology, oxidative stress and cardiovascular biology and expertise in key research areas for this application. I have published several peer reviewed articles that focus on the molecular mechanisms of ROS-mediated cellular dysfunction, with specific interests on the identification of cellular targets, modification and intracellular organelle dysfunction endothelial cells during oxidative stress. Further, my research has been well appreciated with several awards that are solely related to pathophysiology of oxidative stress. My earlier research on ischemiaireperfusion model studies identified that mitochondrial oxidative stress play a crucial role in organ failure. While, I started exploring permeabilized cells as a model for studying the direct effect of agonist-induced mitochondrial dysfunction, I developed a rapid filtration technology to assess apoptotic protein release from the mitochondria. I laid the ground work for the proposed research by interrogating how paracrine and autocrine derived ROS alters mitochondrial Ca^{2+} homeostasis leading to mitochondrial functional changes and vascular dysfunction. My lab is well equipped and has standardized methods for analyzing live tissue imaging by multi photon confocal system. Recently, I have been awarded the state-of-the-art intravital multiphoton confocal microscopy instrumentation grant to translate cell culture findings to relevant *in vivo* settings. My laboratory is engaged in several mitochondrial related projects including, the effects of oxidants on mitochondrial Ca^{2+} handling, the molecular identification of mitochondrial Ca^{2+} uptake, and the role of MCU complex in mitochondrial Ca^{2+} overload during pathophysiological conditions.

B. Positions and Honors:

Positions and Employment

1993-1995	Junior Res. Fellow:	GATE-liT (Graduate Aptitude Test Examination-Indian Institute ofTechnology)
1996-1998	Senior Res. Fellow:	CSIR (Council of Scientific and Industrial Research -India)
1999-2003	Post Doctoral Fellow:	Department of Pathology, Anatomy and Cell Biology, Kimmel Cancer Institute, Thomas Jefferson University, Philadelphia, PA 19107
2003-2008	Imaging and Morphology Director:	Institute for Environmental Medicine (IFEM), University of Pennsylvania, PA 19104
2006-2008	Res. Assistant Professor:	Department of Cancer Biology, University of Pennsylvania, Philadelphia, PA 19104
2008-present	Assistant Professor:	Department of Biochemistry, Temple University, Philadelphia, PA 19104 Member, Center for Translational Medicine, Cardiovascular Research Center, Fels Institute of Cancer Research and Molecular Biology

Honors:

1997 **Shakuntala Amir Chand Award for Bio-Medical Young Scientist** (Indian Council of Medical

	Research-India)
1998-1999	**Dr. S.S. Misra Memorial Award** (National Academy of Medical Sciences-India).
2000	**Indian National Science Academy Invited for Young Scientist Award Presentation.**
2001	**Keystone Symposia Scholarship Award** (Molecular Mechanisms of Apoptosis-USA)
2006	**Mid-Atlantic AHA study section committee** (Basic cell and molecular biology) University
2006	**University Research Foundation Scholar** (University of Pennsylvania)
2007	**Shared Instrumentation Grant** (NIH (NCRR) Multiphoton Microscopy)
2007	**Austrian Science Fund** (Grant reviewer)
2010	**Shared Instrumentation Grant** (NIH (NCRR) Multiphoton Microscopy).
2013	**Young Bioenergeticist Award-Biophysical Society** (Cell and Nature Cell Biology papers)

Journal Reviewer: **Proceedings of the National Academy of Sciences of the USA (PNAS)**
Journal of Clinical Investigation
Molecular and Cellular Biology
Journal of Biological Chemistry
Cardiovascular Research
Antioxidants and Redox signaling
American Journal of Respir. Crit. Care Med
Free Radical Biology and Medicine
Cell Calcium
Circulation Research ..... several others

Membership: ASBMB (American Society for Biochemistry and Molecular Biology)
FASEB (Federation of American Societies for Experimental Biology).
AAAS-(American Association for the Advancement of Science)
ASM-(American Society for Microbiology)

C. Selected Peer-Reviewed Publications (out of 86 total)

Most relevant to the current application

1. **M. Madesh#,** B.J. Hawkins, T. Milovanova, C.D. Bhanumathy, S.K. Joseph, S.P. RamachandraRao, K. Sharma, T. Kurosaki and A.B. Fisher. Selective role for superoxide in $InsP_3R$-mediated mitochondrial dysfunction and endothelial apoptosis. ***The Journal of Cell Biology****.170:*1 079-90, 2005. PMCID: PMC2171541.

2. **B.J. Hawkins, L.A. Solt, I. Chowdhury, A.S. Kazi, M. R. Abid, W.C. Aird, M.J. May, J.K. Foskett, and M. Madesh#.** GPCR Ca'+-linked Mitochondrial Reactive Oxygen Species are Essential for Endothelial/Leukocyte Adherence. ***Molecular and Cellular Biology*** 27:7582-7593,2007. PMCID: PMC2169045.

3. S.S. Roy*, M. **Madesh***, E. Davies, B. Antonsson, N. Danial and G. Hajnoczky. Bad targets the permeability transition pore independent of Bax or Bak to switch between $ca^{''}$ -dependent cell survival and death. ***Molecular Cell*** 33:377-88, 2009. PMCID: PMC2662194.

4. **M. Madesh#**, Wei-Xing Zong, Brian J. Hawkins, Subbiah Ramasamy, Thilagavathi Venkatachalam, Partha Mukhopadhyay, Patrick J. Doonan, K.M. Irrinki, M. Rajesh, P. Pacher, and C.B. Thompson. Execution of superoxide-induced cell death by the proapoptotic Bcl-2-related proteins Bid and Bak. ***Molecular and Cellular Biology*** 29:3099-3112,2009. (**Chosen for Faculty of 1000**) PMCID: PMC2681999.

5. M.D. Levin, M.M. Lu, N.B. Petrenko, B.J. Hawkins, D. Lang, P.T. Buckley, J. Jochems, C.F. Spurney, J.T. Jacobson, C.B. Brown, L. Huang, L. Huang, F. Beermann, **M. Madesh**, J.H. Eberwine, JA Epstein and vv **.** Patel. Melanocyte-like cells in the heart and pulmonary veins contribute to atrial arrhythmia triggers. Journal of Clinical Investigation 119(11):3420-3436 2009. (**Commentary in Science, Nature Medicine and Science Translational Medicine**). PMCID: PMC2769175

6. B.J. Hawkins, M.D. Levin, P.J. Doonan, N.B. Petrenko, C.W. Davis, V.V. Patel and **M. Madesh#**. Mitochondrial

complex II prevents hypoxic but not calcium- and proapoptotic Bcl-2 protein-induced mitochondrial membrane potential loss. ***Journal of Biological Chemistry*** 285(34):26494-26505, 2010. (**Highlighted in NAVBO, North American Vascular Biology Organization**) PMCID: PMC2924085

7. Hawkins, B.J., Irrinki, K., Lien, Y., Wang, Y., Bhanumathy, *CD.*, Subbiah. R, Mallilankaraman, K., R, Richie, M.F., Soboloff. J., Baba, Y., Kurosaki, T., Joseph, S.K., Gill, DL, **M. Madesh#** . S-glutathionylation activates STIM1 and alters mitochondrial homeostasis. ***The Journal of Cell Biology.***190:391-405, 2010. PMCID: PMC2922639

8. K.M. Irrinki, K. Mallilankaraman, R.J. Thapa, H.C. Chandramoorthy, F.J. Smith, N.R Jog, RK. Gandhirajan, S.G. Kelsen, SR. Houser, M.J. May, S. Balachandran, **M. Madesh#**. Requirement of FADD, NEMO and BAXIBAK for aberrant mitochondrial function in TNFa-induced necrosis. ***Molecular and Cellular Biology*** 31 :3745-3758, 2011. PMID 21746883.

10. Soboloff, J, Brad Rothberg, **M. Madesh**, and Gill, D.G. STIM proteins: dynamic calcium signal transducers. ***Nature Reviews Molecular Cell Biology.*** 13: 549-565, 2012. *(**Cover Page**).* PMID: 22914293.

11. Mallilankaraman, K., DoonanP.J., Cardenas, C., Harish C. Chandramoorthy, Marioly Muller, Russell Miller, Nicholas E. Hoffman, Rajesh Gandhirajan, Jordi Molg6, Morris Birnbaum, Brad Rothberg, Don-On Daniel Mak, J. Kevin Foskett and **M. Madesh#**. MICU1 is an Essential Gatekeeper for MCU-Mediated Mitochondrial Ca'+ Uptake That Regulates Cell Survival. ***Cell*** 151: 630-644, 2012. PMID: 23101630.

12. Mallilankaraman, K., Cardenas, C., Doonan, P.J., Chandramoorthy, H.C., Irrinki, K.M., Golenar, T., Csordas, G., Madireddi, P., Yang, J., Muller, M., Miller, R, Kolesar, J.E., Molgo, J., Kaufman, B., Hajnoczky, G., Foskett, J.K, and M. **Madesh#**. MCUR1 is an Essential Component of Mitochondrial $ca^{"}$ Uptake that Regulates Cellular Metabolism. ***Nature Cell Biology*** 14: 1336-1343, 2012. (**Highlighted in Nature Reviews Molecular Cell Biology**) PMID: 23178883.

13. S. Mancarella, S. Potireddy, Y. Wang, H. Gao, RK. Gandhirajan, M. Autieri, R Scalia, Z. Cheng, H. Wang, **M. Madesh**, SR. Houser, and D.L. Gill. Targeted STIM Deletion Impairs Calcium Homeostasis, NFAT Activation, and Growth of Smooth Muscle. ***FASEB Journal*** 27(3):893-906, 2013. PMID: 23159931.

14. Gandhirajan, R M., Meng, S., Chandramoorthy, H.C., Mallilankaraman, K., Mancarella, S., Gao, H., Razmpour, R, Yang, X.F., Houser, S.R, Chen, J., Koch, W.J., Wang, H., Soboloff, J., Gill, D.L. and **M. Madesh#**. Blockade of NOX2 and STIM1 signaling limits lipopolysaccharide-induced vascular infiammation. ***Journal of Clinical Investigation*** 123:887-902, 2013. (**Commentary by Michael Matthay**) **Featured article (JCI Impact) in Feb 2013 issue** (**Chosen for Faculty of 1000**). PMID: 23348743.

15. BA Miller, J.F. Wang, E. Gao, J. Song, X-Qian Zhang, W.J. Koch, **M. Madesh**, K. Mallilankaraman, 1. Gu, S-jen Chen, K. Keefer, I.H. Laszkiewicz, K. Conrad, A.M. Feldman, and J.Y. Cheung. The Second Member of Transient Receptor Potential-Melastatin (TRPM2) Channel Family Protects Hearts from Ischemia-Reperfusion Injury. ***American Journal of Physiology*** 304(7):H1 01 0-22, 2013. PMID: 23376831.

16. J.M. Duran, CA Makarewich, T.E. Sharp, 1. Starosta, F. Zhu, N.E. Hoffman, Y. Chiba, **M. Madesh**, RM. Berretta, H. Kubo, and S.R Houser. Bone-Derived Stem Cells Repair the Heart after Myocardial Infarction Through Transdifferentiation and Paracrine Signaling Mechanisms. ***Circulation Research*** 113(5):539-552. 2013. PMID:23801066.

Additional recent publications of importance to the field (in chronological order)

1. C. White, C. Li., J. Yang, N.B. Petrenko, **M. Madesh,** C. B. Thompson and J. K. Foskett. The endoplasmic reticulum gateway to apoptosis: Bcl-XL modulation of the $InsP_3$ receptor. ***Nature Cell Biology*** 7:1021-8, 2005. (**Chosen for Faculty of 1000**) PMCID: PMC2893337

2. RG. Jones, T. Bui, C. White, **M. Madesh**, C.M. Krawczyk, T Lindsten, S. Kubek, KA Frauwirth, L.Y. Wang, B.J. Hawkins, S.J. Conway, H. Roderick, MD. Bootman, H.Shen, J. K. Foskett, and C.B. Thompson. Bax and Bak regulate T cell proliferation through control of ER $ca^{"}$ homeostasis. ***Immunity*** 27: 268-280, 2007. (**Chosen for Faculty of 1000**) PMCID: PMC2714273

3. J. Soboloff, **M. Madesh**, DL Gill. Sensing cellular stress through STIM proteins. ***Nature Chemical Biology***. 7:488-492, 2011. PMID: 21769090.

4. Vagnozzi RJ, Gatto GJ Jr, Kallander LS, Hoffman NE, Mallilankaraman K, Ballard VL, Lawhorn BG, Stoy P, Philp J, Graves AP, Naito Y, Lepore JJ, Gao E, **M. Madesh** and Force T. Inhibition of the Cardiomyocyte-Specific Kinase TNNI3K Limits Oxidative Stress, Injury, and Adverse Remodeling in the Ischemic Heart. ***Science Translational Medicine***. 16;5(207):207ra141, 2013. PMID:24132636

5. Huang ZM, Gao E, Fonseca FV, Hayashi H, Shang X, Hoffman NE, Chuprun JK, Tian X, Tilley DG, **M. Madesh**, Lefer DJ, Stamler JS, Koch WJ. Convergence of g protein-coupled receptor and s-nitrosylation signaling determines the outcome to cardiac ischemic injury. ***Science Signaling***. 29;6(299):ra95, 2013. PMID:24170934.

* Equal contribution.
[#]Corresponding author

D. RESEARCH SUPPORT:

Current Research Support:

NIH-R01HL086699-06 (Muniswamy, PI) 03/15/2014-03/14/2019

Coordination of pathophysiologic endothelial cell signaling by ROS.

Major Goals:
1. Characterize the role of MICU1 in EC mitochondrial Ca2• homeostasis.
2. Study the role of MICU1 in EC signaling and function.
3. Determine the role of 02.- transmitted InsP3R Ca2• signaling in nuclear infiammatory signaling.

NIH-R01HL 119306-01 (Muniswamy, PI)

Inhibition of MCUR1-MCU mediated mitochondrial Ca^{2}• uptake prevents I/R injury. 04/01/2014-03/31/2018

Major Goals:

1. Investigate the regulatory role of MCUR1 in MCU activity.
2. Investigate the mechanistic role of MCUR1 in cardiomyocyte mitochondrial function.
3. Investigate whether MCUR1 downregulation inhibits mitochondrial dysfunction during I/R injury.

<u>Completed Research</u>

NIH-R01 HL086699-05 (PI: Madesh Muniswamy) 09/01/2008-03/14/2014
Coordination of pathophysiologic endothelial cell signaling by ROS.
Major Goals:
1. Examine the role of $InsP_3Rs$ in *Oi"* mediated Ca^{2}• signaling.
2. Determine the coordination of Oi"-induced $InsP_3R$ Ca^{2}• signaling and mitochondrial function in vasculature.
3. Determine the role of *Oi"* transmitted $InsP_3R$ Ca^{2}• signaling in nuclear inflammatory signaling.

1 R21 HL 109920-02 (Madesh Muniswamy/Jonathan Soboloff (MPI) 08/01/2011-05/31/2014
ROS-dependent STIM1 activation and Ca^{2}• entry in lung infiammation
Major Goals:
1) Characterize the role of ROS-induced Ca2+ signaling in endothelial cell activation
2) To examine whether targeting the STIM1 control vascular inflammatory responses.