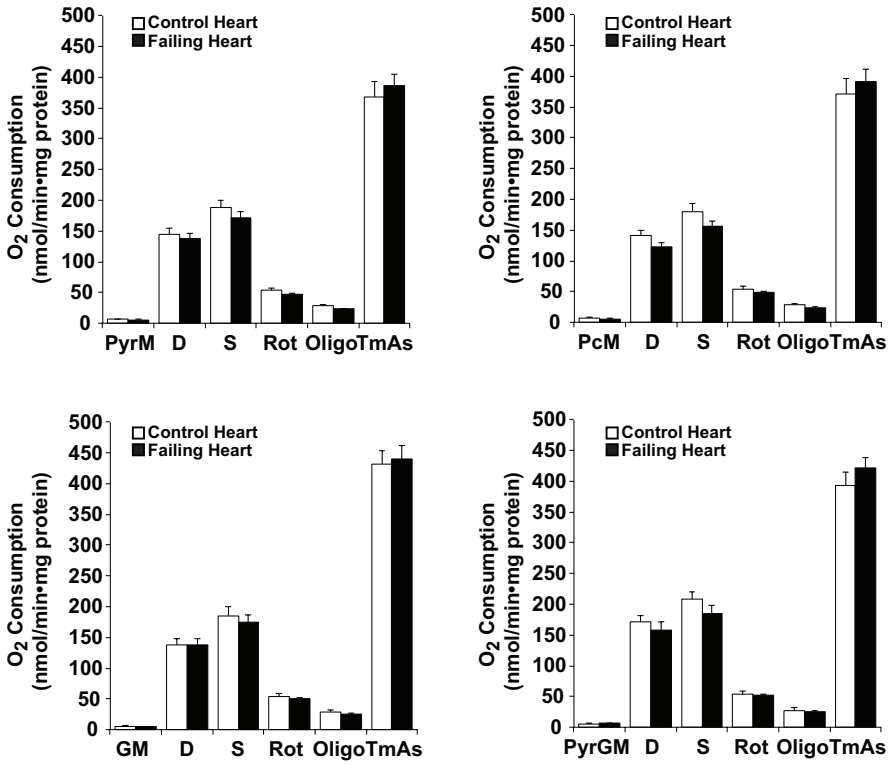
Supplemental Data

Heart failure–induced activation of phospholipase iPLA$_2\gamma$ generates hydroxyeicosatetraenoic acids opening the mitochondrial permeability transition pore

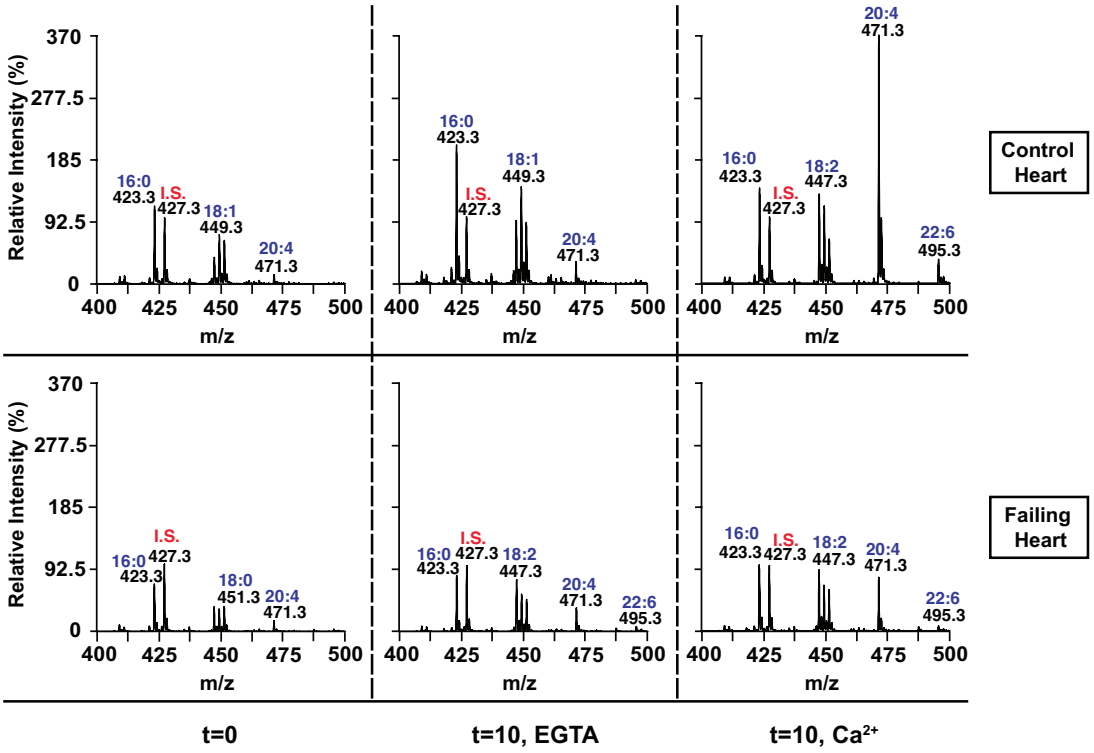
Sung Ho Moon[1], Xinping Liu[1], Ari M. Cedars[1,*], Kui Yang[1,*], Michael A. Kiebish[1,*], Susan M. Joseph[2,*], John Kelley[1,*], Christopher M. Jenkins[1], Richard W. Gross[1,3,4,5]

From the [1]Division of Bioorganic Chemistry and Molecular Pharmacology, the Department of Medicine,
the [2]Cardiovascular Division, the Department of Medicine
the [3]Department of Developmental Biology,
and the [4]Center for Cardiovascular Research, the Department of Medicine,
Washington University School of Medicine, Saint Louis, Missouri 63110
and the [5]Department of Chemistry, Washington University, Saint Louis, Missouri 63130

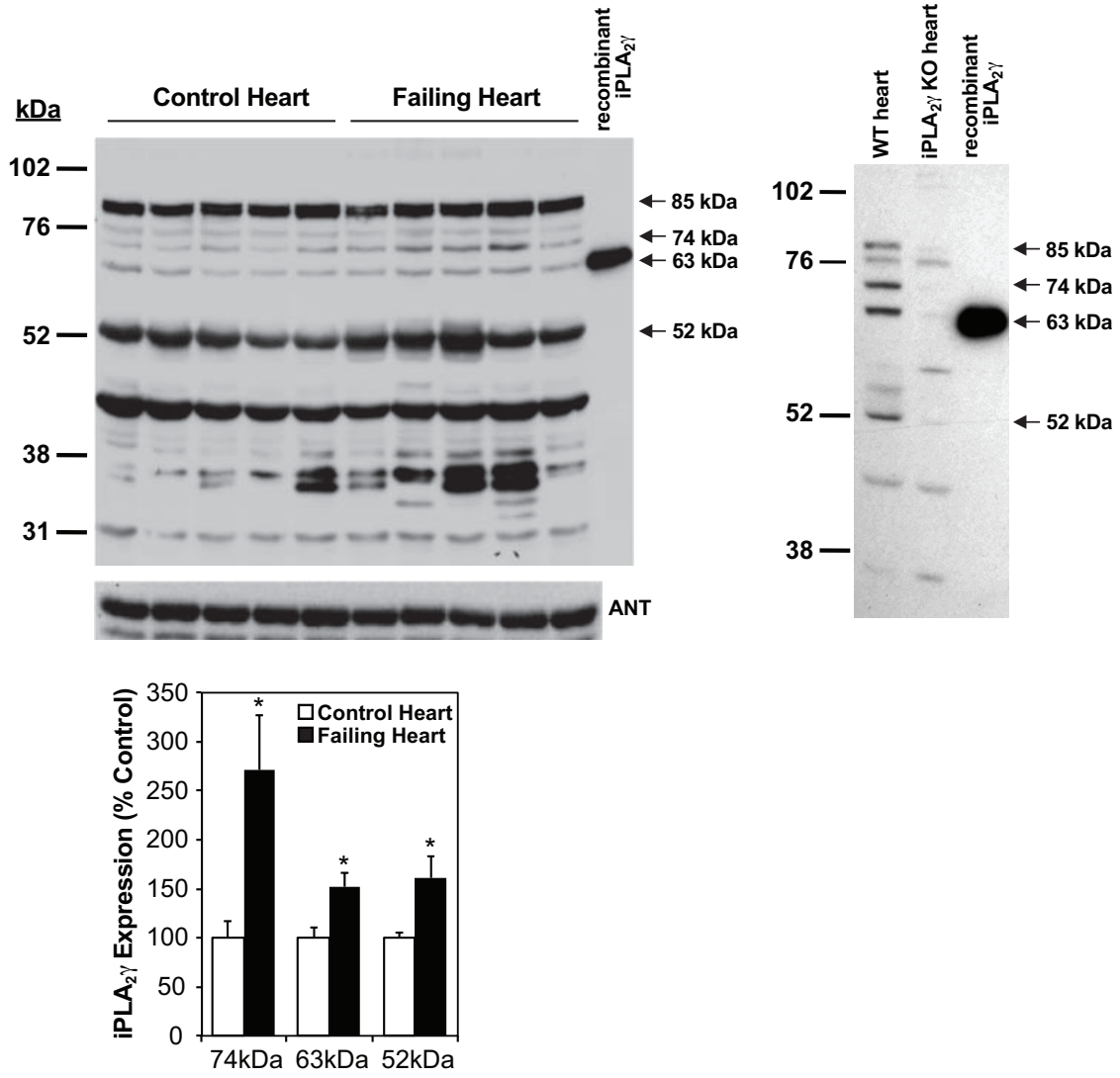
Address correspondence to:
Richard W. Gross, M.D., Ph.D.
Division of Bioorganic Chemistry and Molecular Pharmacology
Washington University School of Medicine
660 S. Euclid Ave., Campus Box 8020
St. Louis, MO 63110
Tel.: 314-362-2690
Fax: 314-362-1402
Email: rgross@wustl.edu.



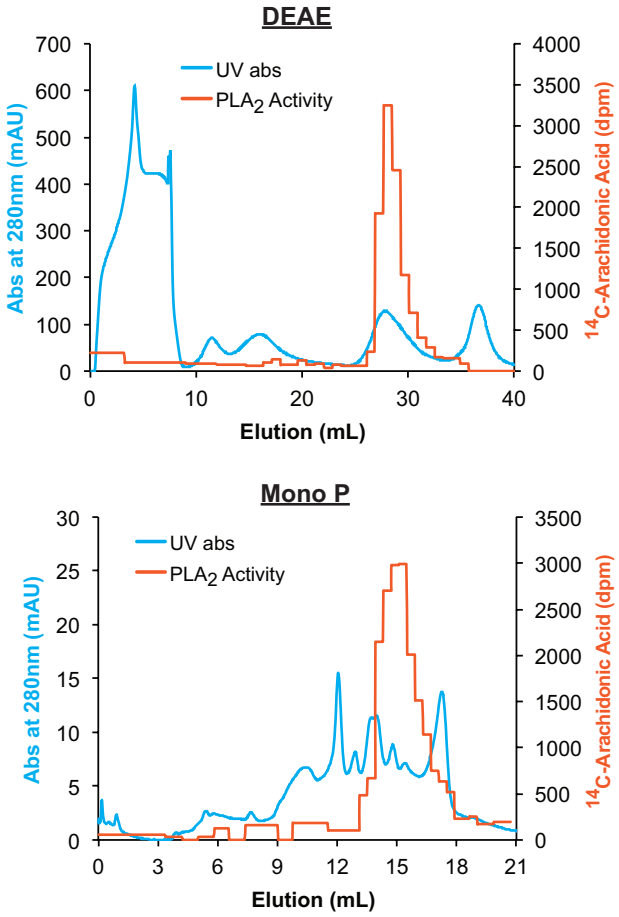
Supplemental Figure 1. High resolution respirometry of human heart mitochondria. Myocardial mitochondria isolated from non-failing (control) (n=11) and failing (n=26) human hearts were placed in ice-cold MiRO5 buffer. High resolution respirometry was performed using 50 µg mitochondria in a 2 ml chamber OROBOROS® Oxygraph 2K at 30ºC. Mitochondrial respiration was initiated by the addition of either 5 mM pyruvate/ 5mM malate (PyrM), 20 µM palmitoylcarnitine /malate (PcM), 10 mM glutamate/malate (GM), or pyruvate/glutamate/malate (PyrGM) [State 2] followed by sequential addition of 1.25 mM ADP (D) [State 3], 5 mM succinate (S) [State 3 Max], 0.5 µM rotenone (Rot), 2.5 µM oligomycin (Oligo) [State 4], 5 µM *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine with 0.5 mM ascorbate (TmAs) and antimycin A (1 µM). All values reflect the subtraction of residual oxygen consumption after addition of antimycin A.



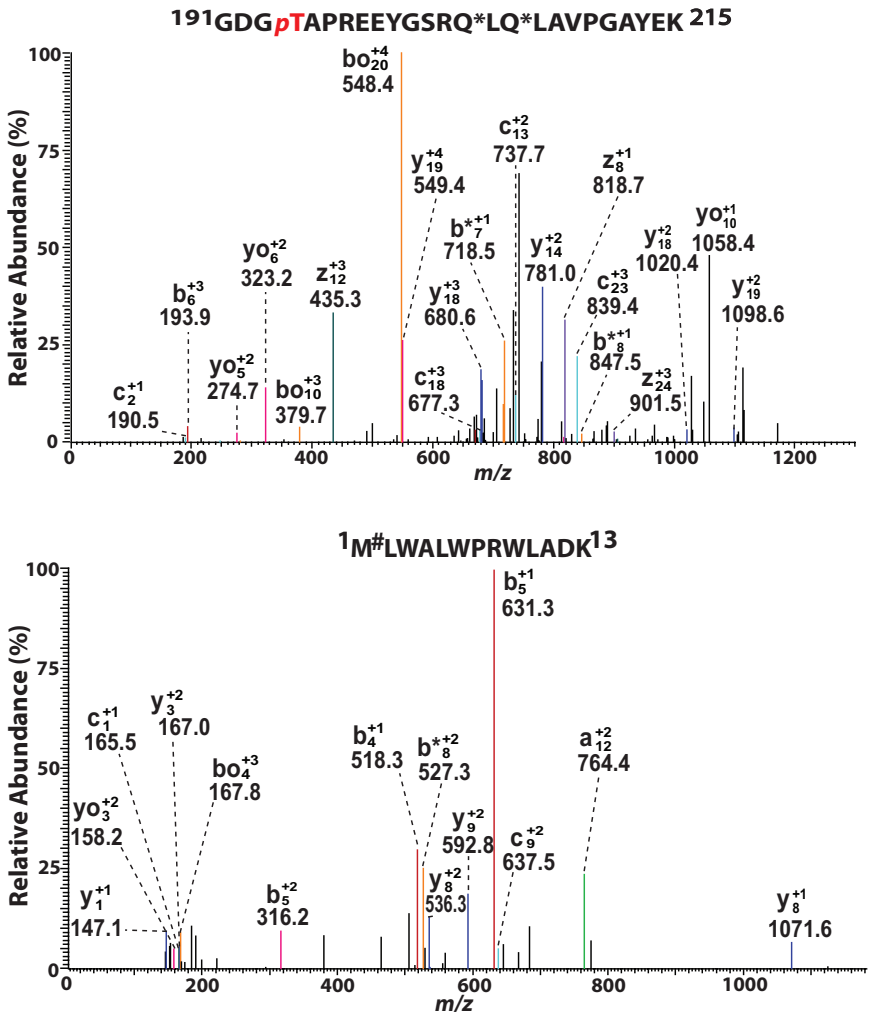
Supplemental Figure 2. Mass spectrometric analysis of fatty acids released from endogenous human heart mitochondrial phospholipids in the absence and presence of Ca^{2+}. Mitochondria from human non-failing (control) and failing hearts were placed in HEPES buffer (10 mM HEPES (pH 7.4) containing 1 mM DTT and 10 % glycerol) prior to brief sonication. Mitochondrial phospholipase activity was initiated by addition of either 2 mM EGTA or 0.6 mM free Ca^{2+} (final concentration) and incubated for 10 min at 35°C. Free fatty acids were extracted into chloroform, derivatized with AMPP and analyzed using a TSQ Quantum Ultra mass spectrometer in the positive ion mode through precursor ion scanning of m/z 183. Representative spectra for AMPP-derivatized fatty acids including palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2), arachidonic acid (20:4) and docosahexaenoic acid (22:6) with internal fatty acid standard (I.S., d_4-16:0 FFA) are shown.



Supplemental Figure 3. Comparison of iPLA$_2$γ isoform expression levels in mitochondria isolated from human non-failing control (n=5) and failing hearts (n=5). Western analysis of the expression levels of multiple iPLA$_2$γ isoforms in human myocardium mitochondria was performed utilizing a polyclonal anti-iPLA$_2$γ antibody (upper left panel). Human recombinant iPLA$_2$γ (63 kDa) and ANT were used as a positive control and a loading control, respectively. For identification of iPLA$_2$γ isoforms, Western analysis of iPLA$_2$γ from murine wild-type and iPLA$_2$γ germline-knockout mouse heart is shown in the upper right panel. The intensities of immunoreactive bands at 74, 63 and 52 kDa identified as isoforms of iPLA$_2$γ were normalized to ANT and compared between control versus failing heart (lower panel). *$p<0.05$.



Supplemental Figure 4. Chromatographic purification of human myocardial mitochondrial phospholipase A_2. Mitochondria (8 mg) isolated from non-failing human myocardium were resuspended in highly basic buffer, sonicated, and centrifuged at 100,000×*g* for 1h. The soluble fraction was then purified by DEAE chromatography utilizing a NaCl gradient (upper panel) followed by chromatofocusing using a Mono P column (pH 6.0-4.0) (lower panel) as described in "Experimental Procedures." Protein eluted from each column was monitored by UV absorbance (280 nm) and active fractions were identified by a radioactive assay for PLA_2 activity as described in "Experimental Procedures".



Supplemental Figure 5. Identification of the purified human myocardial mitochondrial PLA_2 as $cPLA_2\zeta$ by mass spectrometric proteomic analysis. Mono Q fractions with high PLA_2 activity were electrophoresed by SDS-PAGE and silver-stained. Regions of the gel corresponding to the band labeled by desthiobiotin-fluorophosphonate were trypsinized and analyzed by mass spectrometry (NanoLC-MS/MS) utilizing a protein sequence database using the SEQUEST algorithm. Mass spectra for two peptides identified as tryptic fragments of $cPLA_2\zeta$ are shown. *p*: phosphorylation; #: oxidation; *: deamidation.