Sample Preparation:

HL-1 cardiomyocyte cells were cultured in T75 flasks pre-coated with gelatin and fibronectin. Claycomb medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin, 100 µg/mL streptomycin, 0.1 mM norepinephrine, and 2 mM L-glutamine was changed daily. Upon confluency, cells were treated with either 1 or 50 µM of GEN for 24 h before harvesting. Control cells were treated with the same volume (<3 µL/mL of medium) of DMSO. Each treatment was performed in triplicate. For each condition, approximately 10^7 cells were collected by trypsinizing.

The cell suspension was washed twice with pH 7.4 PBS and centrifuged at 300 xg for 3 min before protein extraction. Protein extraction was achieved with a two-stage hydrophilic/hydrophobic solubilization protocol. Water-soluble proteins were first extracted by ultrasonication for 5 min in 1 mL hydrophilic extraction solution containing 10 mM Tris-HCL (pH 8.0), 0.5 mM Pefabloc, 5 mM magnesium acetate, and 0.01% Triton X-100. Samples were centrifuged at 17 000 x g for 20 min which the supernatant was designated as the hydrophilic protein fraction. The resulting pellet was further washed twice with 0.5 mL of hydrophilic extraction solution then dissolved with 0.5 mL hydrophobic extraction solution containing 10 mM Tris-HCL (pH 8.0), 7 M urea, 2 M thiourea, 0.5 mM Pefabloc, 5 mM magnesium acetate, and 4% CHAPS. After centrifugation at 17 000 x g, the supernatant was collected as the hydrophobic protein fraction. Total protein concentration of both hydrophilic and hydrophobic protein fractions was determined using the RC-DC protein assay kit (Bio-Rad, Hercules, CA, USA) according to the instructions from the manufacturer. Bovine serum albumin (Thermo Scientific, Rockford, IL, USA) was used as the protein assay standard.

Gel Preparation:

For the hydrophilic protein fraction, 300 µg of protein was brought up to a volume of 400 µL (1:3 or greater dilution) with rehydration buffer (8 M urea, 4% CHAPS, 10% glycerol, and 0.3% dithiothreitol). Eight microliters of IPG buffer (Bio-Rad) were added before loading the sample onto an 18-cm pH 4-7 IPG ReadyStrip (Bio-Rad). For the hydrophobic protein fraction, 200 µg of protein were loaded using the same procedure. Each IPG strip was rehydrated in an Immobiline DryStrip tray (GE Healthcare, Piscataway, NJ, USA) for 16 h. IEF was performed using a Multiphor II electrophoresis system (GE Healthcare) at 20 ^{o}C. The IEF voltage was programmed to increase to 500

V linearly within 1 min, followed by a linear increase to 3500 V over 5 h, and then maintained constant at 3500 V for 17.5 h. Proteins were reduced by submerging the IPG strips in 3 mL equilibration buffer (6 M urea, 30% v/v glycerol, 2% w/v SDS, and 24 mM Tris-HCl pH 6.8) supplemented with 2% w/v dithiothreitol for 15 min. Alkylation was performed by submerging the strips in 3 mL equilibration buffer supplemented with 2.5% w/v iodoacetamide and a trace of bromophenol blue for 5 min. The strips were then loaded onto a 13.5% polyacrylamide SDS-PAGE gel (18 cm x 20 cm x 1 mm). The second dimension of electrophoresis was performed at a constant current of 40 mA per gel for 4.5 h in a Protean II XL 2-D Multi-Cell system (Bio-Rad).

A MS-compatible silver stain protocol was used to visualize gel spots. Briefly, gels were first fixed in 40% v/v ethanol and 10% v/v acetic acid for 30 min, followed by sensitization in 30% v/v ethanol, 0.2% w/v sodium thiosulphate, and 7% w/v sodium acetate for 30 min. After gels were washed three times with Nanopure water (Barnstead, Garner, NC) for 5 min, a 20-min staining was carried out in a 0.25% w/v silver nitrate solution. Gels were washed twice with Nanopure water for 1 min each and visualized with 0.0074% formaldehyde in a 2.5% w/v sodium carbonate solution. After visualization, gels were preserved in 1.5% w/v EDTA. In all steps, a total volume of 250 mL of solution was used per gel.