Supporting Information

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SI Methods

Materials. DMEM and FBS were purchased from Invitrogen. p38 MAPK inhibitor SB203580 was obtained from Calbiochem. Antibodies against phospho-p38 MAPK (Thr180/Tyr182), phospho-p44/42 MAPK (ERK1/2) (Thr202/Tyr204), and phospho-MKK3/6 (Ser189/207), as well as antibodies against p38, p38α, ERK, and JNK were obtained from Cell Signaling Technology. Antibody against phospho-JNK (Thr183/Tyr185) was obtained from Promega. Antibody against TAB1 was obtained from Santa Cruz Biotechnology. Antibody to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was obtained from R&D Systems. Adenoviruses expressing dominant negative p38α and dominant negative p38β were generously provided by Yibin Wang (University of California, Los Angeles).

Immunoblotting. Cultured cardiomyocytes were lysed with cell lysis buffer (Cell Signaling Technology). Cell lysates of equal protein concentration were used for SDS/PAGE and the gel was then transfered to Immobilon PVDF membranes (Millipore). The membranes were blocked with Odyssey Blocking buffer (Li-Cor) for 1 h and incubated with primary antibodies at 4 °C overnight. The blots were washed, incubated with the IRDye 700CW Donkey Anti-Rabbit IgG or IRDye 800CW Donkey Anti-Mouse IgG for 1 h, finally scanned, and analyzed using the Odyssey Infrared System (Li-Cor).

Immunoprecipitation. The overnight cultured rat adult cardiomyocytes were incubated with vehicle, Con-LC (20 μg/mL) or AL-LC (20 μg/mL) for 15 min. After the treatments, cells were quickly washed with cold PBS and harvested by using a cell lysis buffer (Cell Signaling Technology) supplemented with 1 mM PMSF. Cell lysates were incubated at 4 °C for 10 min followed by centrifugation at 13,000 × *g* for 15 min. The supernatants containing equal protein concentrations were incubated with 2 μg of antibody against TAB1 (Santa Cruz Biotechnology) at 4 °C for 3 h with rotation. The protein G Sepharose beads preblocked with BSA (Santa Cruz Biotechnology) were added into the mixtures and incubated with rotation at 4 °C for another hour. The protein G Sepharose beads were washed four times with lysis buffer and one time with PBS. The precipitates were boiled with 40 μL of loading buffer and subject to Western blot analysis with anti-p38 or anti-TAB1.

TUNEL Assay. After treatment with vehicle, Con-LC, or AL-LC for 48 h, cultured cardiomyocytes were fixed with 4% paraformaldehyde solution for 1 h at room temperature and permeabilized with 100% methanol for 30 min at −20 °C. The TUNEL assay was performed by using In Situ Cell Death Detection Kit (Roche) according to the manufacturer's instruction. Nuclei were counterstained with DAPI (Vector Laboratories). TUNEL-positive cells and total cell number per view were counted and recorded under fluorescent microscope linked with computer.

Caspase 3/7 Assay. Caspase 3/7 activity was measured by using the Caspase-Glo 3/7 Assay kit according to manufacturer's instruction (Promega). Briefly, 100-μg proteins of each sample were mixed with 100 μL of caspase-Glo 3/7 reagent for 1 h at room temperature in an orbital shaker (Shaker Orbit 300, Labnet International, Inc.) at a speed of 300–500 rpm. Following the reaction time, the luminescence was determined with a luminometer (Turner Biosystems). The results were expressed as fold changes with an average of nonfailing human hearts set as 1-fold.

In vivo LC Infusion. DNp38αTG mice (kindly provided by Yibin Wang) and wild-type littermates (male, 8 weeks old) were first injected with Con-LC or AL-LC (300 μg per mouse) through the tail vein. Mice were then anesthetized with sodium pentobarbital (65 mg/kg body weight, i.p.), and an incision was made on the ventral surface of the neck. A sterile mouse jugular catheter was inserted 5 mm into the jugular vein and secured with ligature. The free end of the catheter was connected to an osmotic minipump (ALZET, model 1007D) that was inserted under the dorsal skin and filled with 5 μg/μL either Con-LC or AL-LC. The LC infusion lasted 7 days at a delivery rate of 12 μL/day (total infused dose 420 μg of LC per mouse over 7 days). On day 7, the mice were euthanized with overdose of pentobarbital (100 mg/kg body weight, i.p.), and the heart tissues were quickly removed and further processed for staining or protein expression analysis. For staining, the heart tissues were fixed in 4% paraformaldehyde, paraffin embedded, and sectioned at 4-μm thick. TUNEL staining was used for the detection of apoptosis, and Congo Red stain was used for the detection of amyloid fibril deposition.

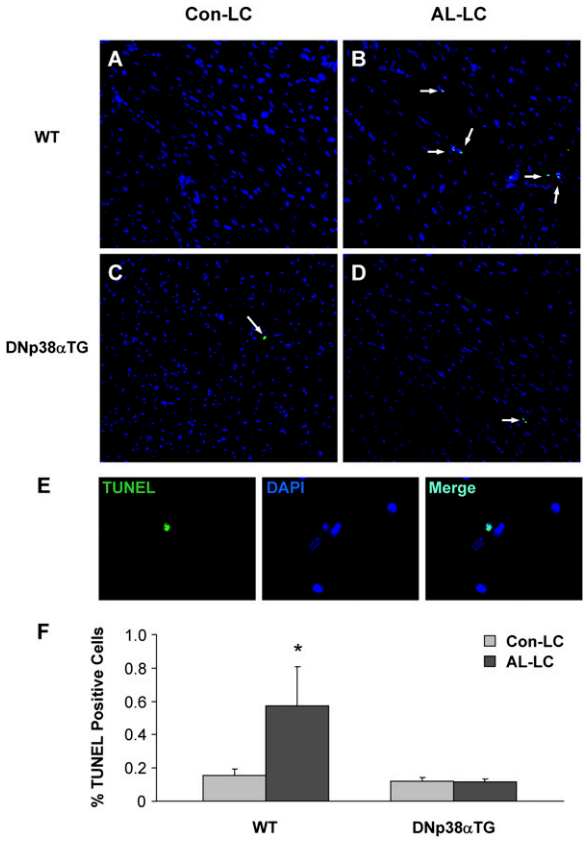

Fig. S1. i.v. administration of AL-LC for 7 days induced cardiac tissue apoptosis (TUNEL-positive cells) in wild-type (WT) mice but not in DNp38αTG mice (*A–D*). Arrowheads indicate TUNEL-positive cells. (*E*) A representative TUNEL positive cell and nuclear counter stain shown in high amplification. (*F*) Bar graph shows percentages of TUNEL-positive cells summarized from three mouse heart tissue sections in each group. Data are means ± SE. *, $P < 0.05$.

Table S1. Correlation of light chains and clinical disease

Abbreviations of proteins	LC isotype	Gender	Dx	Description of clinical manifestations
96–100	κ1	M	MM	No amyloid
00–161	κ	M	MM	No amyloid
00–131	κ1	F	AL	Multisystem with cardiac predominant
00–127	λ3	M	AL	Multisystem with cardiac predominant
99–145	λ2	M	AL	Multisystem with cardiac predominant
00–112	κ1	M	AL	Multisystem with cardiac predominant
01–091	λ1	F	AL	Multisystem with renal predominant
02–131	λ3	M	AL	Multisystem with cardiac predominant
01–052	λ	F	AL	Multisystem with cardiac predominant
01–003	λ1	M	AL	Multisystem with soft tissue predominant

Dx, disease type. Note that all clinical manifestations were defined at the time of urine sample collections. Patients 01–091 and 01–003 were found to have no evidence of cardiac involvement at the time of sample collections. 00–131, 00–127, 99–145, 02–131, and 01–052 were from patients with various degree of cardiac involvement, and the cardiac involvement was predominant as compared to the involvement of other organs.

Table S2. Patient demographics

Characteristics	Nonfailing hearts	AL hearts
Number (male/female)	6 (2/4)	4 (2/2)
Age	63.5 ± 1	54.3 ± 3.1
Heart weight	382 ± 29	515 ± 29
LVEDD, cm	na	38.7 ± 2.8

LVEDD, left ventricle end diastolic diameter. Heart tissues were collected from donor hearts or from explanted hearts of patients with AL cardiomyopathy. All tissues from donor and transplant patients were collected in cold and oxygenated Wisconsin cardioplegic solution immediately after explantation. Failing hearts were obtained from the heart failure program at Massachusetts General Hospital. Nonfailing hearts were purchased from National Disease Research Interchange (NDRI) supported by the National Institutes of Health. Tissues received as nonfailing hearts from donors were found to be not suitable for transplantation due to lack of a suitable recipient. All organ donations were obtained with family approval. Donor hearts were used if no macroscopic, laboratory, or instrumental signs of cardiac diseases were present. All procedures related to using human tissue were reviewed and approved by Institution Review Board (IRB) committee at Massachusetts General Hospital.