Supplementary Table S1. Clinical and Biochemical Characteristics of Patients at Diagnosis of Immunoglobulin Light Chain Amyloidosis or Multiple Myeloma

					Biochemical source and characteristics								*Cardiac parameters*				
Code	*Gender, age*	*Cardiac stage*[a]	*Diagnosis*	*Organ involved*[b]	*Recombinant*	*BJ*	*Germline gene*	*Deduced MW (kDa/pI)*	*Serum λ FLC (mg/L)*	*κ/λ FLC ratio*	*Proteinuria (g/24 h)*	*Creatinine (mg/dL)*	*NT-proBNP (ng/L)*	*cTnI (ng/ml)*	*IVS (mm)*	*PW (mm)*	*EF (%)*
H6	M, 74	III	AL	H		X	*IGLV6-57*	23.2/5.37	683	0.009	0.45	0.73	4300	0.2	15	15	42
H7	M, 45	III	AL	H	X	X	*IGLV1-51*	22.3/6.15	477	0.01	0.33	0.98	8882	0.16	19	19	45
H18	M, 69	III	AL	H		X	*IGLV3-19*	22.7/5.88	509	0.01	0.82	0.97	3839	0.34	21	18	61
MM2	F, 71		MM	—		X	*IGLV3-19*	22.8/5.61	6130	0.001	0.52[c]	2.07	42[d]	0.007	9	9	65
MM4	M, 65		MM	—		X	*IGLV2-23*	22.7/7.72	1140	0.001	0.12[c]	0.89	201	n.a.	10	10	64
MM7	M, 48		MM	—		X	*IGLV2-23*	22.8/8.20	573	0.01	1.87[c]	0.84	14.5	0.003	10	10.5	67

Reference ranges: serum λ FLC <26.3 mg/L, κ/λ ratio 0.26–1.65; serum creatinine <1.18 mg/dL in men, <1.02 mg/dL in women; NT-proBNP (42) <332 ng/L; BNP, <50 ng/L; cTnI <0.04 ng/ml.

[a]According to Gertz *et al.* (19).

[b]According to the International Consensus Panel criteria (19).

[c]Entirely constituted by BJ proteins.

[d]BNP (ng/L).

AL, immunoglobulin light chain amyloidosis; cTnI, cardiac troponin I; EF, ejection fraction; F, female; FLC, free light chains; H, heart; IVS, interventricular septum; BJ, Bence Jones; M, male; MM, multiple myeloma; n.a., not available; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; pI, isoelectric point; PW, posterior wall.

Supplementary Table S2. Effect of CQ and PBT2 on the Pharyngeal Toxicity Induced by the Natural Bence Jones and Recombinant Immunoglobulin Light Chain from the Same Cardiac Amyloid Patient

Treatment	*Pharyngeal pumping (Pumps/min ± SE)*
Vehicle	233.2 ± 1.5
H7-BJ	209.5 ± 1.6[a]
H7-r	212.4 ± 1.0[a]
H7- BJ + CQ	235.2 ± 1.2
H7- BJ + PBT2	238.0 ± 1.4
H7-r + CQ	237.0 ± 1.0
H7-r + PBT2	233.3 ± 1.7

Human monoclonal amyloidogenic cardiotoxic LC obtained as natural Bence Jones (H7-BJ) and recombinant (H7-r) from the same cardiac amyloid patient were administered to worms (100 worms/100 μl) at 100 μg/ml in 10 m*M* PBS, pH 7.4, in the absence or presence of 25 μ*M* CQ or 2 n*M* PBT2. Control worms received vehicle alone (Vehicle). After incubation for 2 h in the absence of OP50 *Escherichia coli*, worms were plated on NGM plates seeded with bacteria. Pharyngeal functionality evaluated 20 h after plating. Pharyngeal pumping is expressed as pumps/minute ± SE.

[a]$p < 0.01$ *versus* vehicle, according to one-way analysis of variance followed by Bonferroni's *post hoc* test.

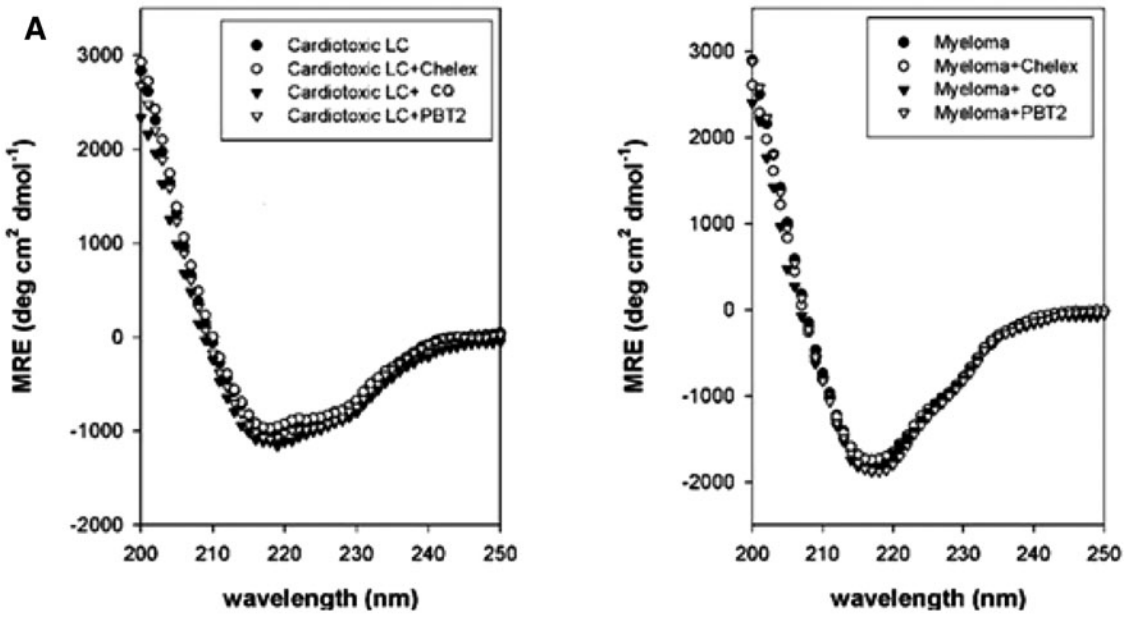
CQ, 5-chloro-7-iodo-quinolin-8-ol; NGM, nematode growth medium.

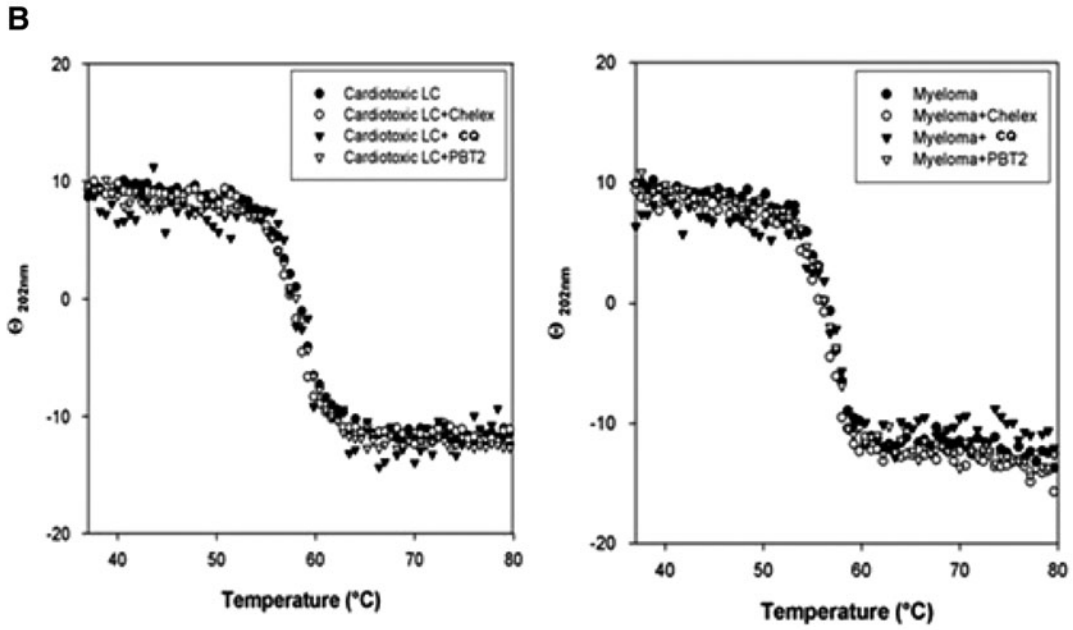
Supplementary Table S3. Clinical and Biochemical Characteristics of Patients Suffering from Immunoglobulin Light Chain Amyloidosis with Severe Cardiac Involvement and Subjected to Endomyocardial Biopsy

Code	*Gender, age*	*Cardiac stage*[a]	*Germline gene*	*Serum λ FLC (mg/L)*	*NT-proBNP (ng/L)*	*cTnI (ng/ml)*	*IVS (mm)*	*PW (mm)*	*EF (%)*
AL1	F, 52	III	*IGLV3-1*	776	4325	0.049	11	n.a.	55
AL2	M,73	III	*IGLV1-44*	59	7182	0.090	15	14	51
AL3	M,71	III	n.a	218	10914	0.100	16	16	50

Reference ranges: serum λ FLC <26.3 mg/L; NT-proBNP (42) <332 ng/L; cTnI <0.04 ng/ml.
[a]According to Gertz *et al.* (19).

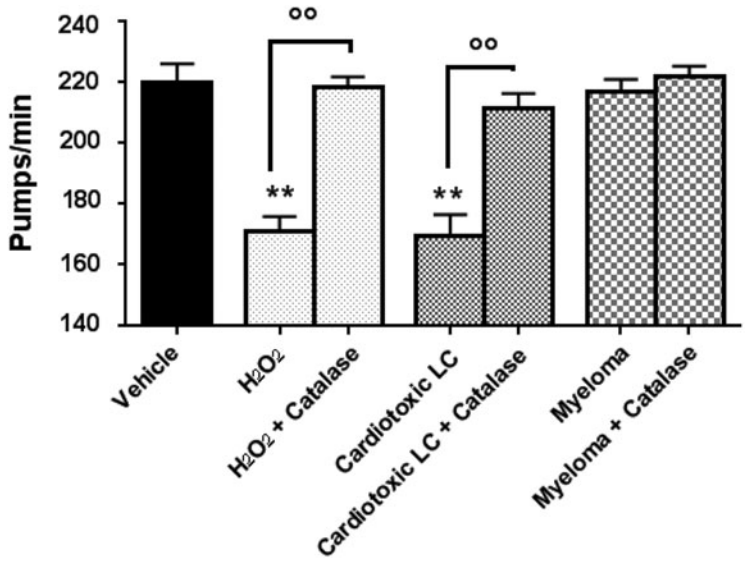
SUPPLEMENTARY FIG. S1. Effect of metal ion chelators on the secondary structure and thermal stability of LC. (A) Far-UV circular dichroism (CD) spectra of cardiotoxic LC (H7-BJ) and myeloma (MM2-BJ) proteins treated or not with 5 mg/ml chelex, 25 μM CQ, or 2 n*M* PBT2. All samples, at protein concentration of 0.4 mg/ml in 50 m*M* sodium phosphate, pH 7.4, were incubated overnight at room temperature. Far-UV CD measurements were performed at 25°C in 50 m*M* sodium phosphate, pH 7.4, with a Jasco J-700 spectropolarimeter (Jasco Europe, Cremella, Italy) using a quartz cuvette with a path length of 1 mm. Scans were conducted from 250 to 200 nm at a speed of 100 nm/min with a spectral band width of 2 nm, a sensitivity of 20 mdegrees, and response time of 1 s. The α-helical and β-sheet content was calculated with K2D, CDSSTR, and CONTIN software applications CD. CD spectra represent the average of 10 scans. Data are shown as mean residue ellipticity (MRE, deg cm^2 $dmol^{-1}$) as function of wavelength. All spectra presented a strong negative band at 216–218 nm. Both cardiotoxic and myeloma proteins had a similar β-sheet content, consistent with the pattern of a typical immunoglobulin, which were not modified by the presence of metal ion chelating agent. **(B)** Melting data were recorded at 202 nm to monitor β-sheet unfolding on sample heating from 37°C to 80°C. Analyses were performed in a 1 mm path length quartz cuvette, temperature slope of 1°C/min, band width of 2 nm, data pitch of 0.2°C, and response time of 2 s. The thermal melt value (Tm) for each condition was calculated at the midpoint of the unfolding transition. No statistical difference among the various conditions tested was observed. No significant perturbations in thermostability were observed in LC after metal ion chelation. BJ, Bence Jones; CQ, 5-chloro-7-iodo-quinolin-8-ol; LC, immunoglobulin light chain.



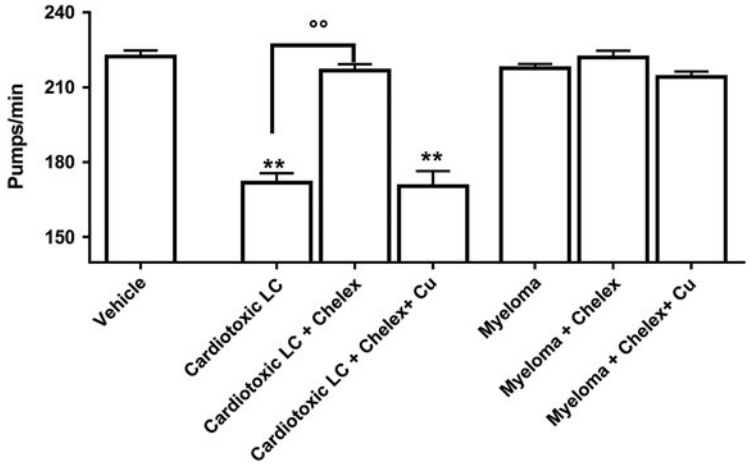


	Tm
Cardiotoxic LC	58.3
Cardiotoxic LC + Chelex	57.7
Cardiotoxic LC + CQ	58.4
Cardiotoxic LC + PBT2	58.3

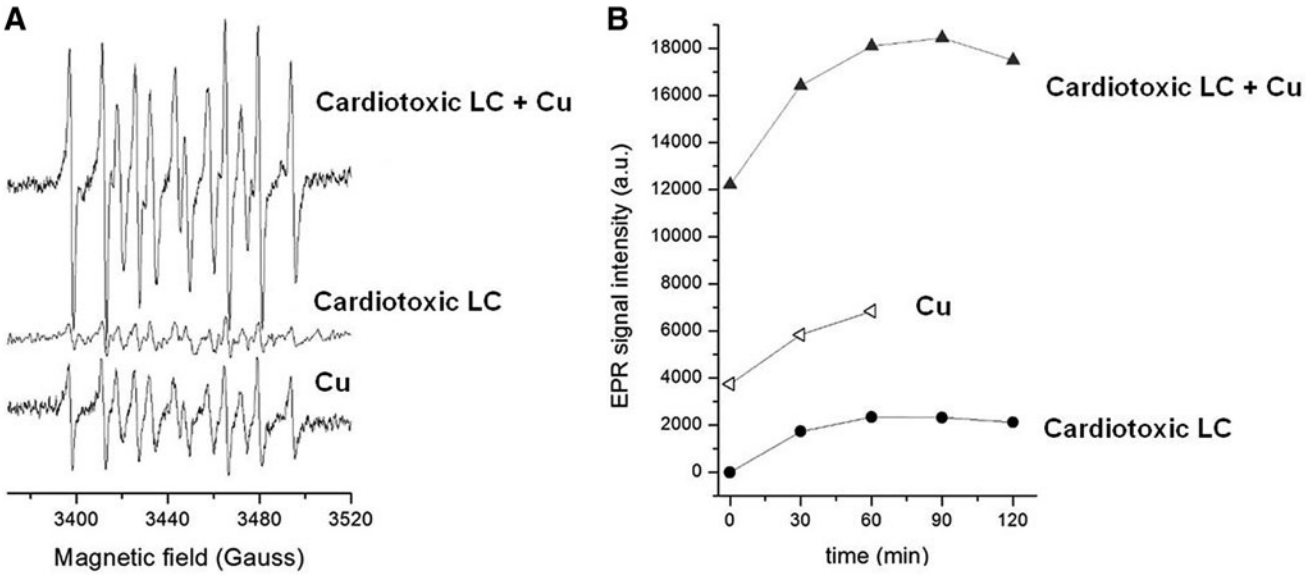
	Tm
Myeloma	56.8
Myeloma + Chelex	56.3
Myeloma + CQ	57.0
Myeloma + PBT2	56.8



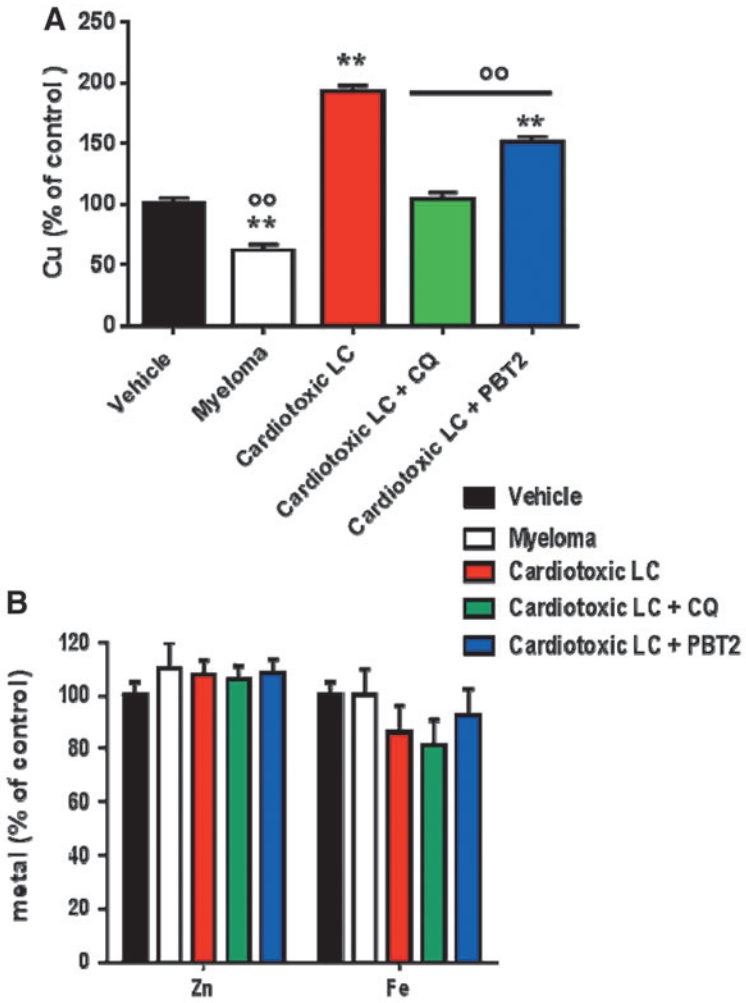
SUPPLEMENTARY FIG. S2. Effect of catalase on cardiotoxic LC-induced pharyngeal dysfunction. Worms were fed for 30 min with 100 μg/ml cardiotoxic LC (H7-BJ) or myeloma (MM2-BJ), or 1 m*M* hydrogen peroxide (used as positive control) previously incubated or not with 100 U/ml catalase for 15 min at room temperature in dark conditions. Control worms received 50 m*M* phosphate buffer, pH 7.0 (Vehicle). Pumping rate as mean pumps/min ± SE (3 independent assays, $n = 30$ worms/assay). $^{**}p < 0.01$ *versus* Vehicle, $^{\circ\circ}p < 0.01$ *versus* the corresponding hydrogen peroxide or cardiotoxic LC not treated with catalase, one-way ANOVA and Bonferroni's *post hoc* test. ANOVA, analysis of variance.



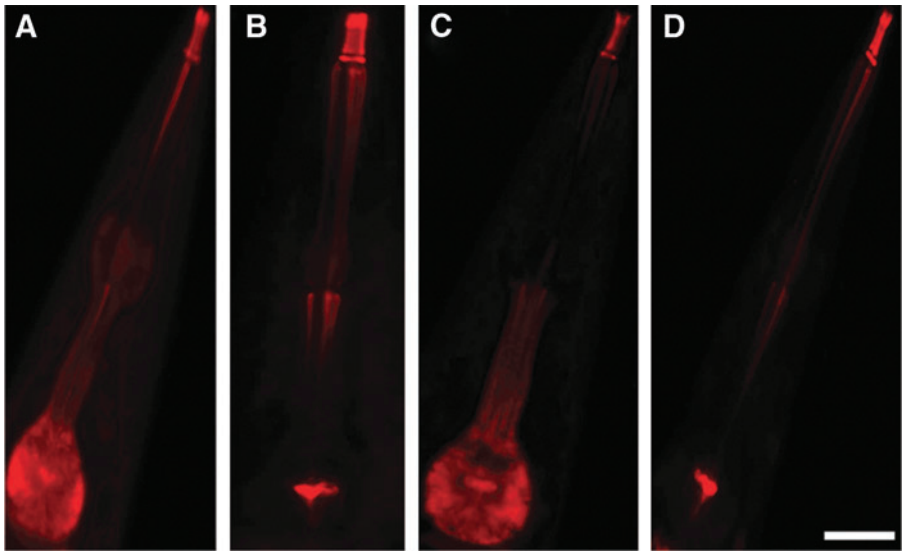
SUPPLEMENTARY FIG. S3. Effect of chelex and copper on LC-induced toxicity. Worms were fed for 2 h with 100 μg/ml cardiotoxic LC (H7-BJ) or myeloma (MM2-BJ) treated or not with 5 mg/ml of chelex and 50 μM copper. Control worms received 50 mM phosphate buffer, pH 7.4, alone (Vehicle). Pumping rate as mean pumps/min ± SE (3 independent assays, $n = 30$ worms/assay). $^{**}p < 0.01$ *versus* Vehicle, $^{\circ\circ}p < 0.01$ *versus* cardiotoxic LC, one-way ANOVA and Bonferroni's *post hoc* test.



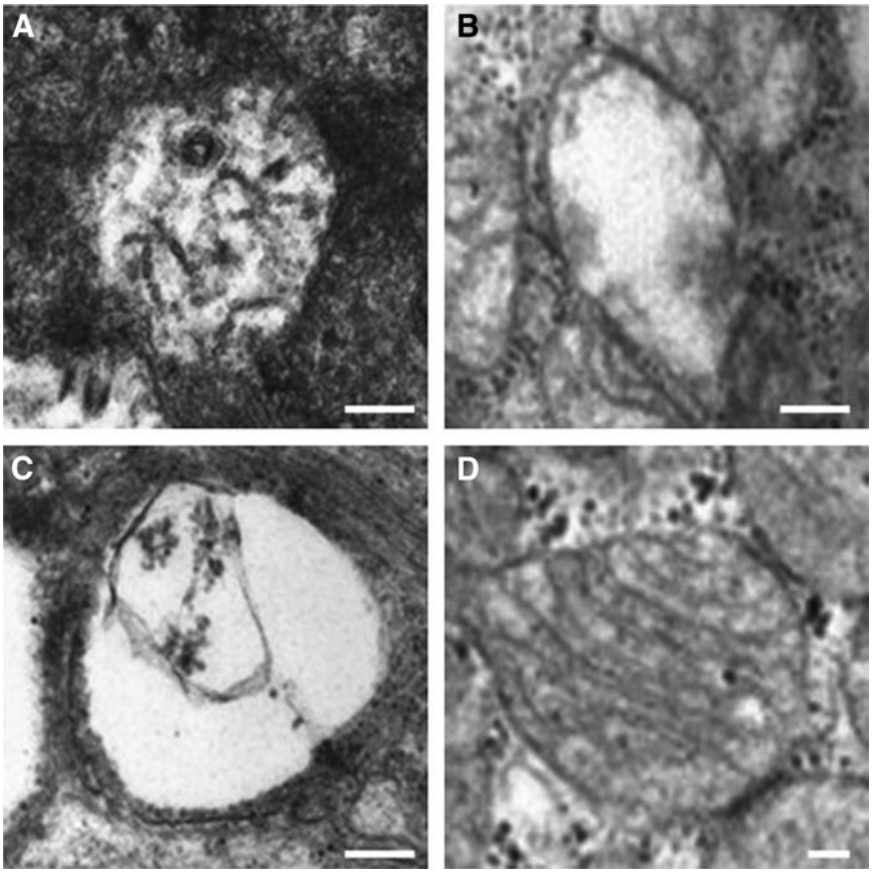
SUPPLEMENTARY FIG. S4. Effect of copper on cardiotoxic LC radical production. (**A**) Electron paramagnetic resonance (EPR) spectra of radical adducts with spin-trap 5-(diethoxyphosphoryl)-5-methyl-1-pyrroline-N-oxide (DEPMPO) obtained in the presence of 60 μM cardiotoxic LC (H7-BJ), 60 μM cardiotoxic LC +30 μM Cu^{2+}, and 30 μM Cu^{2+} only. (**B**). Relative intensity and time dependence of the EPR signals reported in A. The presence of Cu^{2+} ions enhances the production of radicals by the cardiotoxic protein.



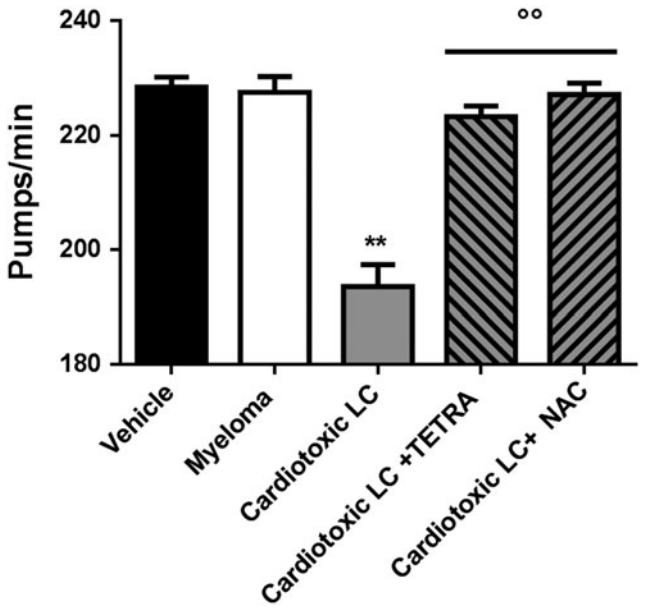
SUPPLEMENTARY FIG. S5. Endogenous metal ion levels in *Caenorhabditis elegans*. Synchronous populations of N2 worms were grown on NGM plates with OP50 *Escherichia coli* as the food source. Worms were collected at L3 larval stage with metal-free water (Sigma Aldrich), pelleted by centrifugation and fed 100 μg/ml cardiotoxic (H7-BJ) or myeloma (MM2-BJ) proteins (100 worms/100 μl, a total of 10.000 worms) in metal-free water, alone or with 25 μM CQ or 2 nM PBT2. Control worms were incubated with metal-free water alone (Vehicle). After 2 h of incubation on orbital shaking, worms were collected with metal-free water, pelletted by centrifugation, and washed in metal-free water three times over a total of 30 min to clear gut content. The worm pellets were dried for 48 h at 60°C and then digested in 6 ml of 65% HNO_3 and 2 ml of 30% H_2O_2. Samples were diluted to a final volume of 10 ml metal-free water for analysis by inductively coupled plasma-atomic emission spectrometry (Varian Simul Vista MPX Radial ICP-OES, Varian Inc.). Data are expressed as the **(A)** copper and **(B)** iron and zinc percentage of control from three biological replicates ± SD. $^{**}p < 0.01$ *versus* Vehicle, $^{\circ\circ}p < 0.01$ *versus* cardiotoxic LC, one-way ANOVA and Bonferroni's *post hoc* test. NGM, nematode growth medium.



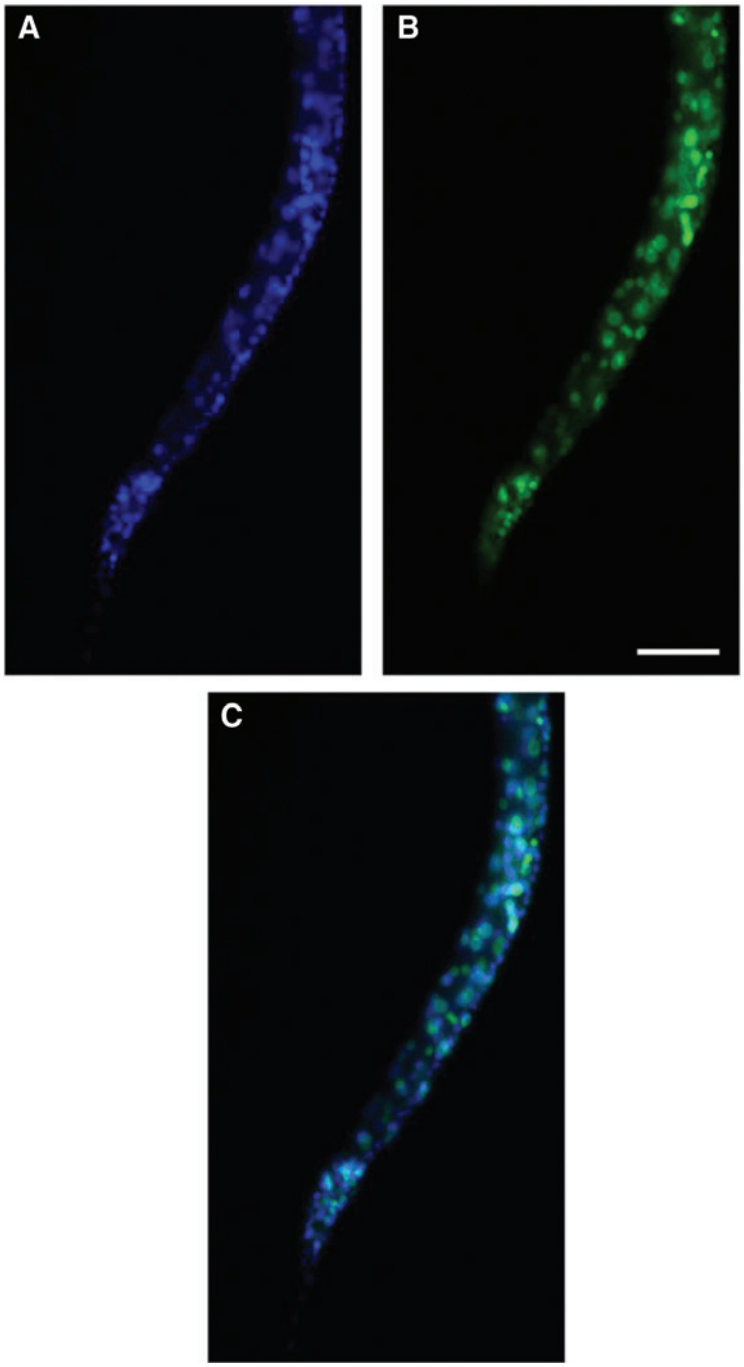
SUPPLEMENTARY FIG. S6. Effect of cardiotoxic LC on pharyngeal mitochondrial membrane potential. Representative images illustrate the tetramethylrhodamine, methyl ester (TMRM) accumulation, which appeared brighter in pharyngeal healthy cells of vehicle-fed worms (**A**), and was lost after the treatment with cardiotoxic LC (**B**), but not myeloma protein (**C**), as indication of depolarization of the mitochondrial membrane potential. A similar effect was observed with H_2O_2, used as positive control (**D**). Scale bar, 50 μm.



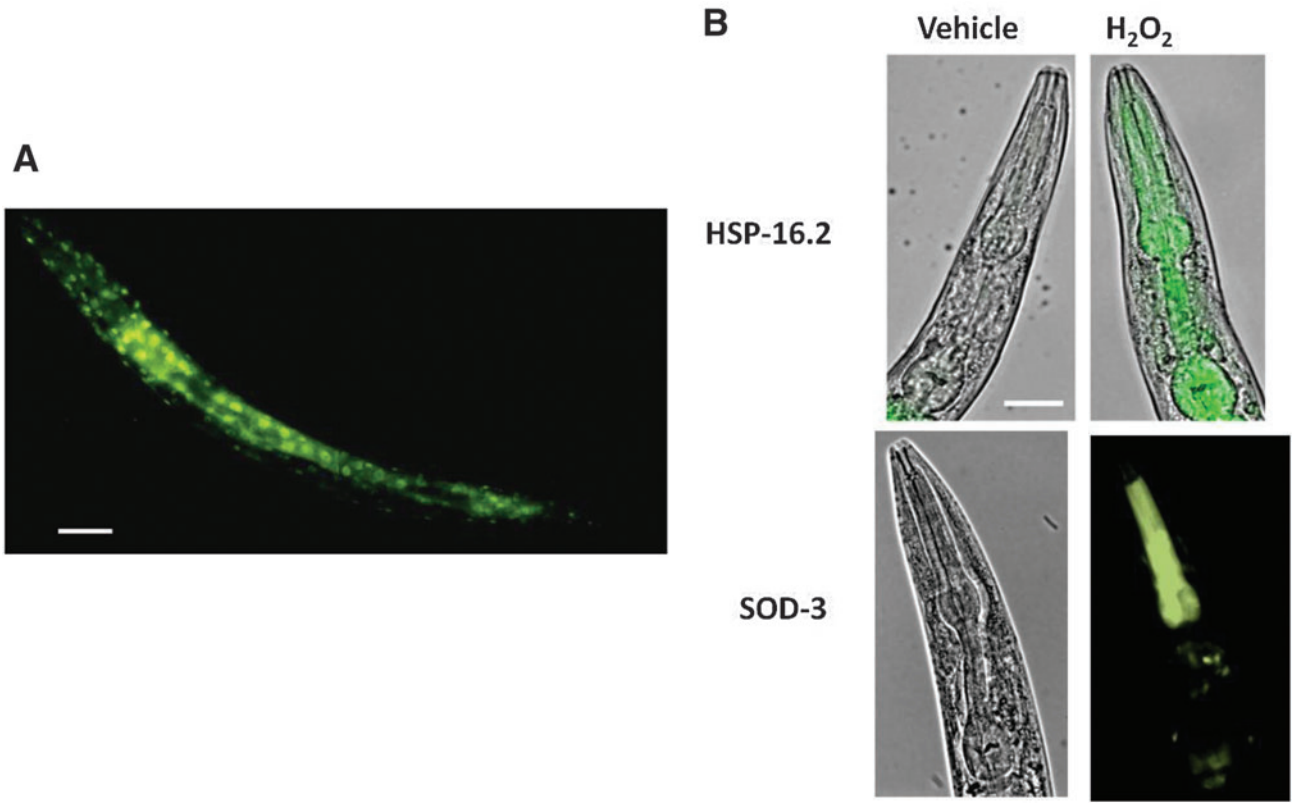
SUPPLEMENTARY FIG. S7. Individual mitochondria from heart muscle tissue of cardiac AL patients (A-C) and of a patient with dilatative cardiomyopathy (D). Ultrastructural details from representative TEM images of individual mitochondria from *insets* of Figure 4. Mitochondria in the myocardium of AL patients show enlarged size and either disruption (**A**) or nearly total loss of cristae (**B, C**). In the myocardium of a patient affected with severe nonamyloid cardiomyopathy (**D**), mitochondria show well-preserved morphology. Scale bar, 200 nm.



SUPPLEMENTARY FIG. S8. Protective effect of TETRA and NAC on the pharyngeal pumping dysfunction caused by cardiotoxic LC. Cardiotoxic LC (H7-BJ) and myeloma (MM2-BJ) in 10 m*M* phosphate-buffered saline, pH 7.4, were administered to worms (100 worms/100 μl) at 100 μg/ml alone or with 50 μ*M* TETRA or 5 m*M* NAC. Control worms received vehicle alone (Vehicle). After incubation for 2 h in the absence of OP50 *E. coli*, worms were plated on NGM plates seeded with bacteria. Pharyngeal pumping was evaluated 20 h after plating and expressed as pumps/minute. $^{**}p < 0.01$ *versus* vehicle and $^{\circ\circ}p < 0.01$ *versus* cardiotoxic LC, according to one-way ANOVA and Bonferroni's *post hoc* test. NAC, *N*-acetylcysteine; TETRA, tetracycline hydrochloride.



SUPPLEMENTARY FIG. S9. Colocalization of nuclear staining and DAF-16::GFP in transgenic TJ356 worms fed cardiotoxic LC. Representative images depict in (**A**) the *blue* fluorescence of nuclear staining and in (**B**) the *green* fluorescence provided by DAF-16::GFP labeling. The nuclear localization of DAF-16::GFP was demonstrated by the colocalization of the two stainings in panel (**C**). Scale bar = 100 μm. GFP, green fluorescent protein.



SUPPLEMENTARY FIG. S10. Effect of hydrogen peroxide on DAF-16 nuclear translocation and HSP-16.2 and SOD-3 pharyngeal expression. Transgenic worms were fed with 1 m*M* H_2O_2 for 30 min. Worms were then plated on NGM agar plates seeded with *E. coli*. **(A)** Representative image of DAF-16::GFP distribution in transgenic TJ356 *C. elegans*. Scale bar = 50 μm. (**B**) HSP-16.2 and SOD-3 expression, as visualized by GFP fluorescence, in transgenic CL2070 and CF1553 worms, respectively. Scale bar = 100 μm. HSP, heat shock protein; SOD, superoxide dismutase.