Regression of pressure-induced left ventricular hypertrophy is characterized by a distinct gene expression profile

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Objective: Left ventricular hypertrophy is a highly prevalent and robust predictor of cardiovascular morbidity and mortality. Existing studies have finely detailed mechanisms involved with its development, yet clinical translation of these findings remains unsatisfactory. We propose an alternative strategy focusing on mechanisms of left ventricular hypertrophy regression rather than its progression and hypothesize that left ventricular hypertrophy regression is associated with a distinct genomic profile.

Methods: Minimally invasive transverse arch banding and debanding (or their respective sham procedures) were performed in C57Bl6 male mice. Left ventricular hypertrophy was assessed physiologically by means of trans-thoracic echocardiographic analysis, structurally by means of histology, and molecularly by means of real-time polymerase chain reaction. Mouse hearts were genomically analyzed with Agilent (Santa Clara, Calif) mouse 44k developmental gene chips.

Results: Compared with control animals, animals banded for 28 days had a robust hypertrophic response, as determined by means of heart weight/body weight ratio, histologic analysis, echocardiographic analysis, and fetal gene expression. These parameters were reversed within 1 week of debanding. Whole-genome arrays on left ventricular tissue revealed 288 genes differentially expressed during progression, 265 genes differentially expressed with regression, and only 23 genes shared by both processes. Signaling-related expression patterns were more prevalent with regression rather than the structure-related patterns associated with left ventricular hypertrophy progression. In addition, regressed hearts showed comparatively more changes in energy metabolism and protein production.

Conclusions: This study demonstrates an effective model for characterizing left ventricular hypertrophy and reveals that regression is genomically distinct from its development. Further examination of these expression profiles will broaden our understanding of left ventricular hypertrophy and provide a novel therapeutic paradigm focused on promoting regression of left ventricular hypertrophy and not just halting its progression.

Supplemental material is available online.

Left ventricular hypertrophy (LVH) affects approximately 25% of adult Americans and is most often associated with pressure overload, as seen with essential hypertension, aortic stenosis, and ischemic heart disease.¹ LVH is highly morbid and is a robust risk factor for cardiovascular mortality.² Yet despite optimal blood pressure control, treated hypertensive patients rarely achieve more than a 15% to 20% reduction in

left ventricular (LV) mass. Furthermore, nearly half of these patients will continue to have LVH progression and increased cardiovascular events, including death.³ Thus there remains a great need to expand our understanding and treatment of LVH.

To date, most experimental and therapeutic approaches addressing pressure-induced LVH have been directed at its progression. Numerous studies have profiled the genomic response to aortic banding at multiple time points,⁴ in different chambers,⁵ and in different sexes.⁶ Collectively, these types of studies have exquisitely detailed and identified mechanistic targets that can be blocked to delay or restrict LVH progression^{7,8} but have not been effective in advancing therapy for existing disease. As opposed to the plethora of studies focusing on LVH development, few studies have attempted to characterize independent features of LVH regression. Friddle and colleagues⁹ first detailed genomic differences with LVH regression using nascent gene array technology after administration and withdrawal from β -adrenergic agonists. Although no significant physiologic differences between groups were discerned, 8 genes unique to the drug removal were identified. Despite only 4000 genes on the array platform, this study revealed that gene expression differences between groups might exist in the absence

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Abbreviations and Acronyms

DAVID	= Database for Annotation, Visualization, and Integrated Discovery
Hpcal1	= hippocalin-like 1
Limma	= linear models for microarray
LV	= left ventricular
LVH	= left ventricular hypertrophy
PCR	= polymerase chain reaction
Plk1	= polo-like kinase 1
SAFE	= Significance Analysis of Functional Expression

of detectable physiologic changes and that array technology might be sensitive enough to highlight these differences.

We and others have demonstrated that removal of a previously placed constrictive aortic band can allow reversal of LVH.¹⁰⁻¹² Similarly, studies of human patients with LVH who undergo aortic valve replacement for aortic stenosis are highly successful in causing LVH regression.¹³ It remains unknown, however, whether LVH regression is truly a unique process and thus a target for focused study and intervention. Accordingly, we sought to determine whether gene expression associated with physiologic LVH regression was not simply the reverse of those genes associated with LVH progression but rather a distinct genomic pattern.

MATERIALS AND METHODS

Surgical Model and Experimental Design

In accordance with both an institutionally approved Institutional Animal Care and Use Committee protocol and National Institutes of Health guidelines, 10-week-old C57Bl6 male mice were randomly assigned to one of 4 groups: sham, band, sham deband, and deband. The 60 mice enrolled were partitioned as follows: 10 in the sham group, 15 in the band group, 13 in the sham deband group, and 21 in the deband group. Mice in the sham and band groups underwent minimally invasive transverse aortic banding, as previously described.¹⁰ Briefly, animals were anesthetized with inhaled isoflurane administered through a facemask. A midline neck incision was used to approach the anterior mediastinum. The transverse arch was identified, and a constrictive band was placed and tightened to the approximate diameter of a 27-gauge needle. The only difference between the sham and band groups was that in the sham group, the constrictive band was not tightened. Adequate placement of the band was verified by means of evaluation of carotid Doppler scans both before and after placement of the aortic band. Adequate banding was accepted when the Doppler velocity ratio doubled from the right to left carotid arteries. Animals in the deband and sham deband groups had initial procedures identical to those for the band and sham groups, respectively. At 4 weeks, the aortic band was removed, heretofore referred to as being debanded.¹⁰ Efficiency of debanding was verified by means of carotid Doppler scanning with normalization of carotid velocities. Animals from the deband and sham deband groups were killed 1 week after the deband procedure for all evaluations except histology, for which animals were killed both at 1 and 4 weeks after debanding.

Transthoracic Echocardiography

Transthoracic echocardiography was performed with the Vevo 660 High Resolution Biomicroscopy System equipped with a 30-Mhz transducer

(Visual Sonics, Toronto, Calif). During examination, mice were anesthetized with 1% to 1.5% inhaled isoflurane. The depth of anesthesia was standardized by recording images at heart rates of 480 to 520 beats/min. Images were recorded in all animals before surgical intervention, at 2 and 4 weeks after banding, and at 1, 2, 3, and 4 weeks after removal of aortic constriction (debanding). Two technicians blinded to the animals' experimental status performed examinations and measurements.

Tissue Procurement

At the time of death, hearts were rapidly excised, and the LV apices were sectioned and flash-frozen in liquid nitrogen for gene array analysis. Additional animals allocated to histologic analysis were perfused with phosphate-buffered saline followed by 10% formalin, fixed overnight in formalin, and then processed for histologic analysis with periodic acid–Schiff staining.¹⁴ Cardiomyocyte cross-sectional area was measured with ImageJ software (version 1.38j; National Institutes of Health, Bethesda, Md).¹⁵

RNA Preparation, MicroArray Process, and Real-time Polymerase Chain Reaction

Semipooled groups of mice were created to minimize variation between mice within a tested group and to enhance detection of variation between experimental groups. For each experimental group, 9 mice were selected from a group of up to 22 based on echocardiographic criteria of both progression with banding (increase in LV mass of >20%) and regression with debanding (decrease in LV mass of >10%). Within these groups, subgroups of 3 animals were randomly pooled so that 3 samples would be created for each experimental group.

LV apices were homogenized in 0.5 mL of ice-cold Trizol solution (Sigma, St Louis, Mo) by using a bead mill homogenizer (Retsch, Newtown, Pa). RNA was then isolated with the standard Trizol procedure, with additional steps for removal of DNA and fibrous tissue. Purity of RNA was verified by using a 260/280 ratio of 1.95 or greater. An Agilent (Santa Clara, Calif) BioAnalyzer 2100 instrument was used to verify RNA integrity for all samples. Five hundred nanograms of total RNA was labeled with Cyanine-5-CTP in a T-7 transcription reaction by using the Agilent Low Input Linear RNA Amplification/Labeling System. Labeled cRNA from samples was then hybridized to Agilent mouse 44k developmental microarray slides in the presence of equimolar concentrations of Cyanine-3-CTP-labeled mouse reference RNA prepared from pools of 1-day-old mouse pups. Real-time polymerase chain reaction (PCR) was performed with Taqman primers and probes (Applied Biosystems International, Foster City, Calif).

Western Blotting

Protein fractions were isolated in ice-cold lysis buffer during Dounce homogenization. Concentrations were determined by using the Bradford assay. Protein fractions were denatured in loading buffer. Thirty micrograms of each sample was then loaded into alternating lanes for gel electrophoresis. Membrane transfer was performed overnight, and rabbit anti-mouse antibody was used to probe for polo-like kinase 1 (Plk1) and hippocalin-like 1 (Hpcal1). Glyceraldehyde-3-phosphate dehydrogenase was used as the loading control.

Statistical Methods

All physiologic data are presented as the mean $\pm$ standard error, except where noted. Real-time PCR data were log-transformed before comparison. All comparisons of physiologic data were performed by using 2-tailed type 3 or type 1 *t* tests. Microarray data (*n* = 12 arrays) were less normalized, and probes were filtered for features having a normalized intensity of less than 30 aFU in either red or green channels. Probes were removed if data were not present in at least 9 of 12 samples. Missing data points were imputed (to facilitate further statistical comparisons) by using the *k* nearest-neighbors algorithm (*k* = 2). Samples were then standardized (μ = 0, σ = 1) using

a custom Perl script (ActiveState Perl 5.8.1, build 807; ActiveState Software, Inc, Vancouver, British Columbia, Canada). Differences in expressed genes were validated by using significance analysis of microarrays with a false discovery rate of 5%.

Linear models for microarray (Limma)¹⁶ was used to model the variation in all data sets and perform the following contrasts: band versus sham, deband versus sham deband, and (deband – sham deband) versus (band – sham). These contrasts were executed by using custom scripts written in the R statistical language and environment (R Version 2.2.1, build r36812). Genes were identified as significant in each contrast with the criteria of a *P* value of .01 or less and a mean fold change of 1.2 or greater.

Unsupervised gene and array clustering was performed for each comparison: band versus sham, deband versus band, and deband versus sham deband. Data for each gene being compared were filtered for 3 instances of a mean fold change of 2 or greater. Data were further adjusted by median centering the genes. Average linkage hierarchic clustering with a centered correlation similarity metric for both genes and arrays was performed with Gene Cluster 3.0 (Michiel de Hoon, University of Tokyo, Human Genome Center, Tokyo, Japan). Data were then visualized with Java TreeView (version 1.0.13).

Gene ontologies of significant genes in each contrast were identified by using the National Institutes of Health–curated Database for Annotation, Visualization, and Integrated Discovery (DAVID) 2007.¹⁷ To facilitate interpretation of gene ontology, functional clustering was performed, and results were rank ordered by using DAVID with enrichment analysis and were further organized according to the number of genes in that cluster relative to the total number of genes identified within a particular contrast. The primary advantage of the DAVID technique is its ability to take large lists of genes and identify the biologic processes and functions that are most important (within the set of significant genes) to the biologic phenomenon under study. Orthogonal filtering of data sets was performed by using the Significance Analysis of Functional Expression (SAFE)¹⁸ with custom R scripts. In this method, also called orthogonal filtering, all genes are first organized based on their gene ontologies or gene ontology categories. Each ontologic category is then evaluated based on the degree of variation of individual genes within that category, regardless of the significance of individual genes. The more randomly the genes within each ontology are distributed, the less likely that that ontology is contributing to the observed differences in the experimental groups. Ontologies are then ranked based on their relative significance to the process under study. Essentially, the entire gene array is used to generate meaningful information about the process under study, rather than just a few dozen genes that are identified as significantly different. Gene ontologies with a *P* value of .1 or less were identified in all contrasts.

RESULTS

Regression of Pressure-induced LVH

Sixty mice were used in this study: 2 died perioperatively, and 2 were excluded with wound infections. Our technique of minimally invasive banding and debanding effectively produced LVH with subsequent regression. Doppler velocity ratios between the right and left carotid arteries verified creation and relief of arch obstruction, as previously demonstrated.¹⁰ Grossly, heart weight/body weight ratios similarly increased with banding and normalized after debanding (Figure 1, A). Echocardiographically, aortic constriction resulted in increased wall thickness, greater chamber dimensions, and significantly greater LV mass (Figure 1, A, and Table E1). One week after band removal, most parameters changed in the direction of baseline, with a favorable trend noted in fractional shortening. Histologically, cardiomyocyte cross-sectional areas increased approximately 20%

after banding and decreased significantly after debanding (Figure 1, B and C). Three common genetic expression markers of ventricular hypertrophy were evaluated by using real-time PCR, including β myosin heavy chain, natriuretic peptide type B, and skeletal muscle α 1-actin (*n* = 6–8), to determine the corresponding molecular changes. All were significantly upregulated in the banded animals and normalized in debanded mice. For example, natriuretic peptide levels increased 65.4 ± 25.4 -fold with banding (sham, 1.0 ± 0.2 -fold; *P* < .05) and decreased with debanding (2.9 ± 0.8 , *P* < .05 vs banding). Taken together, these data comprehensively demonstrate a reproducible model to study mechanisms independently associated with LVH regression.

Identification and Clustering of Significant Genes

To minimize variation in for our array experiments, we used echocardiography to select 9 animals from a larger starting cohort of mice for each group (*n* = 11–22). The sensitivity of the array technology demanded that we study the best representative animals for each group. Furthermore, at the time of these studies, it was cost-prohibitive to do individual arrays for each animal. Nevertheless, changes in LV mass index were not significantly different between the full and array data sets (ie, Δ percentage Band [total, *n* = 17] $35.2\% \pm 4.3\%$ compared with Band [array, *n* = 9] $42.6\% \pm 5.0\%$ [*P* = .29] and the Δ percentage Deband [total, *n* = 22] $-9.8\% \pm 1.9\%$ compared with Deband [array, *n* = 9] $-15.1\% \pm 1.6\%$ [*P* = .09]). After preliminary filtering and standardization, a total of 14,693 distinct transcripts were identified in our heart tissue and analyzed with Limma. In the regression contrast (deband vs band) we identified 255 differentially expressed genes: 92 were upregulated, and 133 were downregulated. Contrasts for LVH progression (band vs sham) and for the regressed state (deband vs sham deband) identified 288 and 727 differentially expressed genes, respectively (Figure 2, A). During LVH progression, there were 158 upregulated and 130 downregulated genes, and in the deband versus sham deband contrast there were 244 upregulated and 483 downregulated (detailed profiles for each contrast provided in Table E2).

Interestingly, we identified only 23 genes that were significantly differentially expressed in both LVH progression and regression. Conversely, 108 genes were distinctly associated with regression. In examining the most differentially upregulated genes in regression, there were 2 cell-cycle control genes, Plk1 and midkine, a myosin peptide not previously associated with the heart; the neural conduction protein Hpcal1; and Pramef12, a protein with homology to a melanoma-associated protein (Figure 2, B). Of these, only midkine has previously been associated with cardiovascular disease.¹⁹ Western blot analysis confirmed the relationship between our gene array and protein expression for Plk1 and Hpcal1 (Figure 2, C). Among the most downregulated genes in regression, only the trends in natriuretic peptide

FIGURE 1. Physiologic and structural evidence of left ventricular hypertrophy reversal. **A**, Heart weight (*HW*)/body weight (*BW*) ratios (*n* = 11–22 per group) and serial echocardiographic measurements showing changes over the depicted time period (*n* = 11–22 per group). **B**, Left ventricular (*LV*) cross-sections stained with periodic acid–Schiff. **C**, Calculated cardiomyocyte cross-sectional areas (*n* = 4 per group). **P* < .05 versus the sham group, †*P* < .05 versus the band group.

precursor type A (*Nppa*) expression were consistent with our prior understanding of LVH, and changes in expression of the remaining genes were unique to this experiment.

Although we identified changes in expression of individual genes, we sought to determine whether LVH regression was associated with a distinct genomic pattern. Indeed, unsupervised clustering of the samples in each comparison showed clear separation of the band and sham groups and of the deband and band groups (Figure 2, *D*). The separation was less distinct in the deband versus sham deband comparison, with 1 sample from the deband group and 1 sample from the sham deband group not clustering appropriately. This observation indicates that the expression patterns of the deband and sham deband groups were less significant than might otherwise have been predicted based on individual

gene differences originally identified by means of Limma. Taken together, the global expression pattern depicted in the unsupervised clustering suggests that differences between groups were not limited to a few hundred genes but rather to several thousand, and those differences were consistent from one group of animals to another.

Gene Expression Profiles Associated With LVH Regression

In the LVH progression analysis gene ontology clustering of our most significant genes showed expected characteristics of hypertrophic stress, including a large focus on actin, microtubules, cytoskeletal integrity, and contractile function, with the remainder including organelle production, transport, and organization (Table 1 and see Figure E1).

FIGURE 2. Gene expression associated with left ventricular hypertrophy regression. A, Venn diagram depicts different and shared genes in each experimental contrast ($P < .01$ and absolute fold change >1.2). B, Most differentially expressed genes in the regression contrast (deband vs band). C, Western Blot for selected proteins identified in the deband versus band comparison. D, Unsupervised gene clustering between groups. B, Band; S, sham; DB, deband; SDB, sham deband.

Similarly designed gene array studies of LVH progression (in mice) have identified these same genomic themes.⁵ In the regression analysis none of these cytoskeletal or contractile clusters were differentially expressed. Instead, there was a predominance of intracellular and extracellular signaling themes. Further breakdown of the functional clusters involved in the regression process indicated that cell growth, morphogenesis, and cell cycle were all highly upregulated, whereas metabolism and protein and nucleotide production were downregulated. Lastly, the regressed state (deband vs

sham deband) had 126 overlapping genes that demonstrated mostly activity in metabolism and in mitochondria and other organelles. This is markedly different from the patterns associated with either LVH progression or regression. This latter observation suggests that although the debanded hearts appear structurally similar to the sham-debanded hearts, they remain genomically distinct.

We next evaluated all 14,693 expressed genes using the orthogonal filtering methodology SAFE to identify significant gene ontologies and gene set enrichment analysis categories that were relevant to the processes of both LVH progression and LVH regression. The SAFE analysis is particularly useful because it gathers information from all genes in the experiment and not merely ontologies characterized by statistically significant genes. In regression, there were 52 gene ontologies and 34 gene set enrichment analysis categories with a P value of .1 or less (Table E3). Importantly, only 5 gene ontologies were identified as common to both LVH progression and regression (Table E4). These data further distinguish LVH regression as an independent process. Taken together, the DAVID and SAFE ontologic analyses corroborate differences identified at the individual gene level

TABLE 1. Gene ontology clustering of significant genes between respective groups, as assessed by using the DAVID 2007 technique and listed in order of prevalence

Band vs sham	Deband vs band	Deband vs sham deband
Organelle production	Protein glycosylation	Cellular metabolism
Intracellular transport	Extracellular signaling	Protein metabolism
Actin/cytoskeletal organization	Glycosaminoglycan binding	Organelle organization
Contractile protein binding	Cell adhesion	Intracellular transport
Nucleotide binding	Cell-cycle control	Ion binding

and demonstrate that LVH regression is associated with a distinct thematic pattern of gene expression.

DISCUSSION

Our results compare favorably with those of a recently published study that detailed gene expression by means of microarray in rats after transverse arch debanding.¹² Although they identified 52 regression-related genes, animals that underwent an operation 2 weeks before heart procurement were compared with animals 24 hours after reoperative chest surgery to remove an inflammatory silk band. Thus many transcripts might actually reflect differences attributed to the perioperative systemic inflammatory response. We purposefully and rigorously designed (cage strategy and circadian cycling of procedures) our study in a minimally invasive murine model to avoid confounding perioperative variables. Furthermore, no quantifiable physiologic data are reported to indicate that LVH regression actually occurred. Additionally, statistical issues evaluating fold changes between groups (differences are expressed in relation to a single sham control group and not between the contrasting groups) are magnified when the essential comparison only used 3 animals, as opposed to 9 animals in each of our groups. Finally, their study used a limited array platform with less than 10,000 transcripts, possibly limiting their scope of observation, especially when compared with the whole-genome platform used in our present study.

Hypertrophy and atrophy have similarly been studied in skeletal muscle, and the processes have much in common with the progression and regression of LVH. For example, atrophy has been previously demonstrated to be an active process with a distinct pattern of gene expression rather than simply the genomic reverse of muscle hypertrophy.^{20,21} Functional genes associated with atrophy and regression involve increased protein degradation, downregulation of adenosine triphosphate production, decreased glycolysis, a decrease in growth protein synthesis, and altered extracellular matrix production, all categories that we identified in regression compared with LVH. Fundamentally, however, muscle atrophy typically describes progression to a disease state, whereas regression of LVH is a return to a normal physiologic state. In this regard, and because of the intrinsic physiologic differences between skeletal and cardiac muscle, we believe that mechanistic inferences must be limited in all but the final pathways that directly result in decreased muscle mass.

Our results must be viewed with several caveats. In this study we have examined in detail the effects of banding at only 1 time point (4 weeks) and for only a discreet period of pressure unloading (1 week). We suspect that the influence of pressure relief when the ventricle is either less (2–3 weeks of banding) or more (≥ 6 weeks of banding) stressed would offer different structural and genomic patterns. We thus are likely witnessing a continuum of change as the heart both negatively and positively remodels. We chose to orga-

nize the array around the 1-week time point because our preliminary data indicated that the effective physiologic and structural reversal of LVH was nearly complete at this time (and far enough away from the surgical procedure), despite differences in gene expression. As demonstrated simply in the Venn diagram, structural reversal did not create a “normal heart,” as assessed genomically. These observations will propel more detailed studies focusing on timing and better understanding of the reversed state. Finally, we acknowledge that this model is really a model that relieves acute pressure overload (banding). Thus parallels to the insidious pressure overload that occurs with typical aortic stenosis in human subjects and its subsequent reversal with aortic valve replacement^{13,22} can only be inferred.

In conclusion, we demonstrate that relief of pressure overload-induced LVH is not simply the reverse of LVH progression but rather a unique process with a specific gene expression profile. Therapeutic approaches to LVH based on enhancing regression, rather than impeding progression, are rarely pursued. Elaborating mechanisms associated with both regression and the regressed state have great potential for identifying novel and clinically relevant strategies for treating patients with LVH.

Clinical Implications

Much attention is given to the study of mechanisms involved with the development of LVH and heart failure. Unfortunately, current strategies have not markedly altered the natural history of this disease. Aortic valve replacement for severe aortic stenosis (ie, removing the band) has clearly demonstrated a favorable effect in cardiac remodeling. Yet there is a large subset of patients with LVH from pressure overload that do not regress and have increased risk of cardiovascular morbidity and mortality. Although an allure exists to jump to elaborate therapies for heart failure (eg, cellular replacement and stem cell injections), a great need exists to identify the underlying mechanisms associated with myocardial recovery that can subsequently be targeted for sole therapy or perhaps in combination with mechanical unloading (aortic valve replacement or ventricular assist). Although LVH experimentation in mice is temporally quite different from that in human subjects, it is nonetheless an important first step in characterizing the genomic signature associated with cardiac remodeling and will enhance future work in myocardial recovery therapy.

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References

1. Levy D, Anderson KM, Savage DD, Kannel WB, Christiansen JC, Castelli WP. Echocardiographically detected left ventricular hypertrophy: prevalence and risk factors. The Framingham Heart Study. *Ann Intern Med.* 1988;108:7-13.

2. Vakili BA, Okin PM, Devereux RB. Prognostic implications of left ventricular hypertrophy. *Am Heart J*. 2001;141:334-41.
3. Koren MJ, Ulin RJ, Koren AT, Laragh JH, Devereux RB. Left ventricular mass change during treatment and outcome in patients with essential hypertension. *Am J Hypertens*. 2002;15:1021-8.
4. Zhao M, Chow A, Powers J, Fajardo G, Bernstein D. Microarray analysis of gene expression after transverse aortic constriction in mice. *Physiol Genomics*. 2004;19:93-105.
5. Wagner RA, Tabibiazar R, Powers J, Bernstein D, Quertermous T. Genome-wide expression profiling of a cardiac pressure overload model identifies major metabolic and signaling pathway responses. *J Mol Cell Cardiol*. 2004;37:1159-70.
6. Weinberg EO, Mirotsoy M, Gannon J, Dzau VJ, Lee RT, Pratt RE. Sex dependence and temporal dependence of the left ventricular genomic response to pressure overload. *Physiol Genomics*. 2003;12:113-27.
7. Caron KM, James LR, Kim HS, Knowles J, Uhler R, Mao L, et al. Cardiac hypertrophy and sudden death in mice with a genetically clamped renin transgene. *Proc Natl Acad Sci U S A*. 2004;101:3106-11.
8. Keys JR, Greene EA, Cooper CJ, Naga Prasad SV, Rockman HA, Koch WJ. Cardiac hypertrophy and altered beta-adrenergic signaling in transgenic mice that express the amino terminus of beta-ARK1. *Am J Physiol Heart Circ Physiol*. 2003;285:H2201-11.
9. Friddle CJ, Koga T, Rubin EM, Bristow J. Expression profiling reveals distinct sets of genes altered during induction and regression of cardiac hypertrophy. *Proc Natl Acad Sci U S A*. 2000;97:6745-50.
10. Stansfield WE, Rojas M, Corn D, Willis M, Patterson C, Smyth SS, et al. Characterization of a model to independently study regression of ventricular hypertrophy. *J Surg Res*. 2007;142:387-93.
11. Gao XM, Kiriazis H, Moore XL, Feng XH, Sheppard K, Dart A, et al. Regression of pressure overload-induced left ventricular hypertrophy in mice. *Am J Physiol Heart Circ Physiol*. 2005;288:H2702-7.
12. Yang DK, Choi BY, Lee YH, Kim YG, Cho MC, Hong SE, et al. Gene profiling during regression of pressure overload-induced cardiac hypertrophy. *Physiol Genomics*. 2007;30:1-7.
13. Taniguchi K, Takahashi T, Toda K, Matsue H, Shudo Y, Shintani H, et al. Left ventricular mass: impact on left ventricular contractile function and its reversibility in patients undergoing aortic valve replacement. *Eur J Cardiothorac Surg*. 2007;32:588-95.
14. Stansfield WE, Tang RH, Moss NC, Baldwin AS, Willis MS, Selzman CH. Proteasome inhibition promotes regression of left ventricular hypertrophy. *Am J Physiol Heart Circ Physiol*. 2008;294:H645-50.
15. Abramoff M, Magelhaes P, Ram S. Image processing with ImageJ. *Biophotonics Int*. 2004;11:36-42.
16. Smyth G. Bioinformatics and computational biology solutions using R and Bioconductor: Limma: linear models for microarray data. In: Gentleman R, Carey V, Huber W, Irizarry R, Dudoit S, eds. *Statistics for biology and health*. New York: Springer; 2005. p. 397-420.
17. Dennis G Jr, Sherman B, Hosack D, Yang J, Gao W, Lane H, et al. DAVID: database for annotation, visualization, and integrated discovery. *Genome Biol*. 2003;4: P3.
18. Barry WT, Nobel AB, Wright FA. Significance analysis of functional categories in gene expression studies: a structured permutation approach. *Bioinformatics*. 2005;21:1943-9.
19. Horiba M, Kadomatsu K, Yasui K, Lee JK, Takenaka H, Sumida A, et al. Midkine plays a protective role against cardiac ischemia/reperfusion injury through a reduction of apoptotic reaction. *Circulation*. 2006;114:1713-20.
20. Glass DJ. Signalling pathways that mediate skeletal muscle hypertrophy and atrophy. *Nat Cell Biol*. 2003;5:87-90.
21. Lecker SH, Jagoe RT, Gilbert A, Gomes M, Baracos V, Bailey J, et al. Multiple types of skeletal muscle atrophy involve a common program of changes in gene expression. *FASEB J*. 2004;18:39-51.
22. Kunadian B, Vijayalakshmi K, Thornley AR, de Belder MA, Hunter S, Kendall S, et al. Meta-analysis of valve hemodynamics and left ventricular mass regression for stentless versus stented aortic valves. *Ann Thorac Surg*. 2007;84:73-8.

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1-day-old mouse pups. Real-time PCR was performed by using Taqman primers and probes (Applied Biosystems International).

Statistical Methods

All physiologic data are presented as the mean $\pm$ standard error, except where noted. Real-time PCR data were log transformed before comparison. All comparisons of physiologic data were performed by using 2-tailed type 3 or type 1 *t* tests. Microarray data (*n* = 12 arrays) were LOESS normalized, and probes were filtered for features having a normalized intensity of less than 30 aFU in either red or green channels. Probes were removed if data were not present in at least 9 of 12 samples. Missing data points were imputed (to facilitate further statistical comparisons) by using the *k* nearest neighbors algorithm (*k* = 2). Samples were then standardized (μ = 0, δ = 1) by using a custom Perl script (ActiveState Perl 5.8.1, build 807). Differences in expressed genes were validated by using significance analysis of microarrays, with a false discovery rate of 5%.

Limma^{E3} was used to model the variation in all data sets and perform the following contrasts: band versus sham, deband versus sham deband, and (deband – sham deband) versus (band – sham). These contrasts were executed by using custom scripts written in the R statistical language and environment (Version 2.2.1, build r36812, release date 2005-12-20.). Genes were identified as significant in each contrast with the criteria of a *P* value of .01 or less and a mean fold change of 1.2 or greater.

Unsupervised gene and array clustering was performed for each comparison: band versus sham, deband versus band, and deband versus sham deband. Data for each gene being compared were filtered for 3 instances of a mean fold change of 2 or greater. Data were further adjusted by median centering the genes. Average linkage hierarchical clustering with a centered correlation similarity metric for both genes and arrays was performed by using Gene Cluster 3.0. (Michiel de Hoon, University of Tokyo, Human Genome Center, 2006). Data were then visualized with Java TreeView software (version 1.0.13).

Ontologies of significant genes in each contrast were identified by using DAVID 2007.^{E4} Functional clustering was performed to facilitate interpretation of gene ontology results, and functional clusters were rank ordered by using DAVID with enrichment analysis and were further organized according to the number of genes in that cluster relative to the total number of genes identified within a particular contrast.

Orthogonal filtering of data sets was performed with SAFE^{E5} by using custom R scripts. Briefly, this test evaluates the significance of a given gene ontology relative to the comparison being studied by using the normality of distribution of all the genes (within that ontology) that are identified within the data set, regardless of the significance of individual genes. All gene ontologies with a *P* value of .1 or less were identified in all contrasts.

References

- E1. Stansfield WE, Rojas M, Corn D, Willis M, Patterson C, Smyth SS, et al. Characterization of a model to independently study regression of ventricular hypertrophy. *J Surg Res*. 2007;142:387-93.
- E2. Abramoff MD, Magelhaes, PJ, Ram SJ. Image processing with ImageJ. *Biophotonics Int*. 2004;11:36-42.
- E3. Smyth GK. Bioinformatics and computational biology solutions using R and Bioconductor: Limma: linear models for microarray data. In: Gentleman RCV, Huber W, Irizarry R, Dudoit S, editors. *Statistics for biology and health*. Vol 19. New York: Springer; 2005. p. 397-420.
- E4. Dennis G Jr, Sherman BT, Hosack DA, Yang J, Gao W, Lane H, et al. DAVID: database for annotation, visualization, and integrated discovery. *Genome Biol*. 2003;4:P3.
- E5. Barry WT, Nobel AB, Wright FA. Significance analysis of functional categories in gene expression studies: a structured permutation approach. *Bioinformatics*. 2005;21:1943-9.

FIGURE E1. Gene ontology clustering of significant genes between respective groups, as assessed by using DAVID 2007. Numbers represent the percentage of our significantly expressed genes in a particular cluster compared with the total number of significant genes. Individual genes might be representative of more than 1 gene ontologic category (thus total percentages will not add to 100%).

TABLE E1. Echocardiographic measurements at each time point for each of the experimental groups

	Sham	Band	Sham deband	Deband
Baseline				
IVSd (mm)	0.63 ± 0.02	0.57 ± 0.02	0.66 ± 0.02	0.56 ± 0.02
LVIDd (mm)	4.2 ± 0.06	4.23 ± 0.08	4.12 ± 0.08	4.14 ± 0.06
LVPWd (mm)	0.56 ± 0.02	0.57 ± 0.02	0.6 ± 0.02	0.56 ± 0.02
IVSs (mm)	0.69 ± 0.02	0.67 ± 0.02	0.73 ± 0.02	0.64 ± 0.02
LVIDs (mm)	3.22 ± 0.09	3.16 ± 0.08	3.15 ± 0.13	3.13 ± 0.1
LVPWs (mm)	0.71 ± 0.02	0.73 ± 0.03	0.72 ± 0.03	0.69 ± 0.01
LVFS (%)	23.1 ± 1.2	25 ± 1.8	23.5 ± 1.9	23.9 ± 1.9
LVEF (%)	46.6 ± 2.1	49.5 ± 2.7	47 ± 3.2	47.7 ± 3.2
LVMass (mg), uncorrected	90.9 ± 4	87.8 ± 2.7	92.9 ± 2.1	83.4 ± 2.8
LVMass (mg), corrected	72.7 ± 3.2	70.2 ± 2.2	74.3 ± 1.7	66.7 ± 2.2
4 wk Banded				
IVSd (mm)	0.62 ± 0.02	0.72 ± 0.02*	0.66 ± 0.02	0.66 ± 0.02*
LVIDd (mm)	4.23 ± 0.14	4.47 ± 0.09*	4.19 ± 0.07	4.44 ± 0.07*
LVPWd (mm)	0.58 ± 0.02	0.67 ± 0.02*	0.59 ± 0.01	0.62 ± 0.01*
IVSs (mm)	0.68 ± 0.02	0.78 ± 0.02*	0.74 ± 0.02	0.73 ± 0.02*
LVIDs (mm)	3.23 ± 0.16	3.58 ± 0.15*	3.23 ± 0.11	3.54 ± 0.11*
LVPWs (mm)	0.71 ± 0.03	0.84 ± 0.04*	0.74 ± 0.03	0.77 ± 0.02*
LVFS (%)	23.6 ± 1.7	20.7 ± 2.3*	23.4 ± 1.5	19.8 ± 1.8*
LVEF (%)	47.1 ± 3	41.7 ± 3.7*	46.9 ± 2.4	40.4 ± 3.1*
LVMass (mg), uncorrected	92.9 ± 5.5	125 ± 4.9*	97 ± 1.8	109.9 ± 3*
LVMass (mg), corrected	74.3 ± 4.4	100 ± 3.9*	77.6 ± 1.4	87.9 ± 2.4*
1 wk Deband				
IVSd (mm)			0.69 ± 0.02	0.58 ± 0.02†
LVIDd (mm)			4.23 ± 0.06	4.37 ± 0.08†
LVPWd (mm)			0.58 ± 0.01	0.58 ± 0.01†
IVSs (mm)			0.73 ± 0.02	0.66 ± 0.02†
LVIDs (mm)			3.28 ± 0.07	3.47 ± 0.13†
LVPWs (mm)			0.72 ± 0.02	0.69 ± 0.02†
LVFS (%)			21.3 ± 1.2	20.3 ± 1.6
LVEF (%)			43.5 ± 2.1	41.4 ± 2.8
LVMass (mg), uncorrected			100.8 ± 2.2	94 ± 3.7†
LVMass (mg), corrected			80.6 ± 1.8	75.2 ± 3†

IVSd, Interventricular septum in diastole; LVIDd, left ventricular internal diameter in diastole; LVPWd, left ventricular posterior wall in diastole and systole; IVSs, interventricular septum in diastole and systole; LVIDs, left ventricular internal diameter in diastole and systole; LVPWs, left ventricular posterior wall in systole; LVFS, left ventricular fractional shortening; LVEF, left ventricular ejection fraction; LVMass, Left ventricular mass. * $P < .05$ versus baseline. † $P < .05$ versus 4 weeks banded.

TABLE E2. A. Genes most differentially expressed in each comparison: Deband versus band groups

Direction (deband)	Accession no.	Symbol	Name	Fold Δ
+	NM_030679	Myh1	Myosin, heavy polypeptide 1, skeletal muscle, adult	2.742
+	U01063	Plk1	Polo-like kinase 1 (<i>Drosophila</i>)	2.475
+	NM_016677	Hpcal1	Hippocalcin-like 1	2.074
+	NM_001012336	Mdk	Midkine	2.044
+	NM_029948	Pramef12	PRAME family member 12	1.979
+	NM_147089	Olfir572	Olfactory receptor 572	1.978
+	AK036567	Mgat5	Mannoside acetylglucosaminyltransferase 5	1.962
+	AK083490	Arfrp1	Adenosine diphosphate-ribosylation factor-related protein 1	1.916
+	NM_009303	Syngr1	Synaptogyrin 1	1.845
+	NM_182991	5330410G16R	RIKEN cDNA 5330410G16 gene	1.730
+	NM_172817	Zfp647	Zinc finger protein 647	1.713
+	NM_007472	Aqp1	Aquaporin 1	1.673
+	AC124532	Calcoco1	Calcium binding and coiled coil domain 1	1.638
+	NM_146201	Zfp553	Zinc finger protein 553	1.628
+	NM_001002272	Tro	Trophinin	1.626
+	NM_011601	Tlm	T lymphoma oncogene	1.596
+	NM_007444	Amd2	S-adenosylmethionine decarboxylase 2	1.550
+	AC155922	Rps6ka5	Ribosomal protein S6 kinase, polypeptide 5	1.538
+	XM_925008	Slc44a5	Solute carrier family 44, member 5	1.538
+	AC161037	Hnrpul1	Heterogeneous nuclear ribonucleoprotein U-like 1	1.530
—	NM_020581	Angptl4	Angiopoietin-like 4	2.550
—	NM_008725	Nppa	Natriuretic peptide precursor type A	2.052
—	XM_621314	Dsp	Desmoplakin	1.928
—	XM_283556	Fer113	Fer-1-like 3, myoferlin (<i>C. elegans</i>)	1.758
—	NM_001013390	Scn4b	Sodium channel, type IV, β	1.701
—	NM_021453	Pga5	Pepsinogen 5, group I	1.651
—	NM_024478	Grpel1	GrpE-like 1, mitochondrial	1.645
—	NM_053176	Hrg	Histidine-rich glycoprotein	1.625
—	NM_019662	Rrad	Ras-related associated with diabetes	1.623
—	NM_171826	Cldnd1	Claudin domain containing 1	1.614
—	NM_133786	Smc4	Structural maintenance of chromosomes 4	1.607
—	NM_023733	Crot	Carnitine O-octanoyltransferase	1.605
—	AC1017091	Lama2	Laminin, α 2	1.601
—	NM_029977	Polq	Polymerase (DNA directed), theta	1.592
—	NM_134072	Akr1c14	Aldo-keto reductase family 1, member C14	1.585
—	NM_012037	Vat1	Vesicle amine transport protein 1 homolog	1.584
—	NM_145406	Slc10a3	Solute carrier family 10, member 3	1.576
—	NM_175277	Bola3	BolA-like 3 (<i>E. coli</i>)	1.555
—	NM_008961	Pter	Phosphotriesterase related	1.554
—	NM_008937	Prox1	Prospero-related homeobox 1	1.551

TABLE E2. *B. Genes most differentially expressed in each comparison: Band versus sham groups*

Direction	Accession no.	Symbol	Name	Fold Δ
+	XM_001002752	Zc3h7b	Zinc finger CCCH type containing 7B	1.884
+	NM_008725	Nppa	Natriuretic peptide precursor type A	1.754
+	NM_007489	Arntl	Aryl hydrocarbon receptor nuclear translocator-like	1.645
+	NM_013468	Ankrd1	Ankyrin repeat domain 1 (cardiac muscle)	1.557
+	NM_015784	Postn	Periostin, osteoblast-specific factor	1.534
+	AC034265	Ankrd23	Ankyrin repeat domain 23	1.530
+	NM_134129	Prpf19	PRP19/PSO4 pre-mRNA processing factor 19 homolog	1.497
+	NM_133357	Krt75	Keratin 75	1.480
+	NM_010480	Hsp90aa1	Heat shock protein 90 kd α , class A member 1	1.454
+	NM_031260	Mov10l1	Moloney leukemia virus 10-like 1	1.445
+	NM_021453	Pga5	Pepsinogen 5, group I	1.445
+	NM_009221	Snca	Synuclein, α	1.439
+	NM_007564	Zfp36l1	Zinc finger protein 36, C3H type-like 1	1.424
+	NM_172621	Clic5	Chloride intracellular channel 5	1.414
+	NM_133744	Ccdc71	Coiled-coil domain containing 71	1.413
+	NM_178701	Lrrc8d	Leucine-rich repeat containing 8D	1.410
+	NM_009007	Rac1	RAS-related C3 botulinum substrate 1	1.394
+	NM_173442	Gcnt1	Glucosaminyl (N-acetyl) transferase 1, core 2	1.388
+	NM_029614	Prss23	Protease, serine, 23	1.378
+	NM_008524	Lum	Lumican	1.365
–	NM_007606	Car3	Carbonic anhydrase 3	2.672
–	NM_030679	Myh1	Myosin, heavy polypeptide 1, skeletal muscle, adult	2.131
–	NM_010005	Cyp2d10	Cytochrome P450, family 2, subfamily d, polypeptide 10	2.051
–	NM_009416	Tpm2	Tropomyosin 2, β	2.016
–	U01063	Plk1	Polo-like kinase 1 (Drosophila)	1.982
–	NM_017370	Hp	Haptoglobin	1.971
–	NM_147089	Olfir572	Olfactory receptor 572	1.925
–	NM_001012336	Mdk	Midkine	1.911
–	NM_011066	Per2	Period homolog 2 (Drosophila)	1.867
–	BC004722	Malat1	Metastasis-associated lung adenocarcinoma transcript 1 (noncoding RNA)	1.859
–	NM_013900	Mfi2	Antigen p97 (melanoma associated)	1.727
–	NM_010404	Hap1	Huntingtin-associated protein 1	1.627
–	NM_177709	Tusc5	Tumor suppressor candidate 5	1.597
–	XM_981394	Rhobtb1	Rho-related BTB domain containing 1	1.586
–	AK083490	Arfrp1	Adenosine diphosphate-ribosylation factor related protein 1	1.567
–	NM_016693	Map3k6	Mitogen-activated protein kinase kinase kinase 6	1.562
–	NM_009920	Cnih2	Cornichon homolog 2 (Drosophila)	1.549
–	NM_025866	Cdca7	Cell division cycle associated 7	1.534
–	NM_001025156	Ccdc93	Coiled-coil domain containing 93	1.524
–	NM_009119	Sap18	Sin3-associated polypeptide 18	1.520

TABLE E2. C. Genes most differentially expressed in each comparison: Deband versus sham deband groups

Direction	Accession no.	Symbol	Name	Fold Δ
+	NM_133777	Ube2s	Ubiquitin-conjugating enzyme E2S	1.967
+	AK036567	Mgat5	Mannoside acetylglucosaminyltransferase 5	1.731
+	NM_029948	Pramef12	PRAME family member 12	1.704
+	NM_011202	Ptpn11	Protein tyrosine phosphatase, nonreceptor type 11	1.620
+	NM_144791	Tor1aip1	Torsin A interacting protein 1	1.507
+	AB009392	Hnrpl	Heterogeneous nuclear ribonucleoprotein L	1.505
+	NM_010890	Nedd4	Neural precursor cell, developmentally downregulation gene 4	1.501
+	NM_028004	Ttn	Titin	1.497
+	NM_001024955	Pik3r1	Phosphatidylinositol 3-kinase, reg. sub., polypeptide 1	1.496
+	NM_008609	Mmp15	Matrix metalloproteinase 15	1.492
+	NM_146087	Csnk1a1	Casein kinase 1, α 1	1.483
+	NM_173364	Zfp445	Zinc finger protein 445	1.480
+	NM_025403	Nola3	Nucleolar protein family A, member 3	1.472
+	NM_152134	Homer1	Homer homolog 1 (Drosophila)	1.469
+	NM_011601	Tlm	T lymphoma oncogene	1.466
+	NM_009652	Akt1	Thymoma viral proto-oncogene 1	1.464
+	NM_146201	Zfp553	Zinc finger protein 553	1.464
+	NM_173028	Vps13a	Vacuolar protein sorting 13A (yeast)	1.464
+	NM_008714	Notch1	Notch gene homolog 1 (Drosophila)	1.447
+	NM_029657	Mgm1	Mahogunin, ring finger 1	1.445
-	NM_020581	Angptl4	Angiopoietin-like 4	1.875
-	NM_001013390	Scn4b	Sodium channel, type IV, β	1.744
-	XM_001001760	Gan	Giant axonal neuropathy	1.671
-	NM_145741	Gdf10	Growth differentiation factor 10	1.651
-	NM_178882	D2hgdh	D-2-hydroxyglutarate dehydrogenase	1.578
-	NM_012037	Vat1	Vesicle amine transport protein 1 homolog	1.572
-	XM_001006025	Rpl19	Ribosomal protein L19	1.558
-	NM_010937	Nras	Neuroblastoma ras oncogene	1.553
-	NM_008937	Prox1	Prospero-related homeobox 1	1.540
-	XM_915717	Ube2e1	Ubiquitin-conjugating enzyme E2E 1, UBC4/5 homolog	1.539
-	XR_001538	Tfb2m	Transcription factor B2, mitochondrial	1.527
-	NM_053176	Hrg	Histidine-rich glycoprotein	1.524
-	NM_025276	Evpl	Envoplakin	1.520
-	AK041640	Zfpn1a4	Zinc finger protein, subfamily 1A, 4 (Eos)	1.517
-	NM_019794	Dnaja2	DnaJ (Hsp40) homolog, subfamily A, member 2	1.516
-	XM_621314	Dsp	Desmoplakin	1.514
-	NM_027777	Pex1	Peroxisome biogenesis factor 1	1.505
-	NM_007666	Cdh6	Cadherin 6	1.502
-	NM_133786	Smc4	Structural maintenance of chromosomes 4	1.501
-	NM_012010	Eif2s3x	Eukaryotic translation initiation factor 2, subunit 3	1.491

TABLE E3. Significant gene ontologies and GSEA categories identified by means of SAFE analysis in LVH regression (excluding those identified in LVH progression)

GO categories		GSEA categories
GO:0015629	Actin cytoskeleton	Actin pathway
GO:0007411	Axon guidance	Adrenergic
GO:0003824	Catalytic activity	Akt pathway
GO:0006968	Cellular defense response	Alk pathway
GO:0004197	Cysteine-type endopeptidase activity	Bcl2 family and reg network
GO:0004519	Endonuclease activity	Carm-er pathway
GO:0005789	Endoplasmic reticulum membrane	Cell cycle
GO:0016251	RNA polymerase II transcription factor	Creb pathway
GO:0004364	Glutathione transferase activity	Death pathway
GO:0006811	Ion transport	Differentiation pathway in PC12 cells
GO:0007254	JNK cascade	Erk5 pathway
GO:0000287	Magnesium ion binding	G α I pathway
GO:0006120	Mitochondrial electron transport, NADH to ubiquinone	Glutamine down
GO:0005554	Molecular function unknown	il2rb pathway
GO:0008137	NADH dehydrogenase (ubiquinone)	IL-4 receptor in B lymphocytes
GO:0003954	NADH dehydrogenase activity	INS
GO:0045786	Negative regulation of cell cycle	IL-4 pathway
GO:0009968	Negative regulation signal transduction	MAPK pathway
GO:0030182	Neuron cell differentiation	Monocyte pathway
GO:0005643	Nuclear pore	mRNA processing
GO:0000786	Nucleosome	mRNA splicing
GO:0005634	Nucleus	mtor pathway
GO:0016491	Oxidoreductase activity	p53 hypoxia pathway
GO:0003755	Peptidyl-prolyl cis-transisomerase	p53 signaling
GO:0007204	Positive regulation of cytosolic [Ca ⁺⁺]	Phosphoinositide-3-kinase pathway
GO:0006470	Protein amino acid dephosphorylation	PIP3 signaling in B lymphocytes
GO:0006412	Protein biosynthesis	Protein modification
GO:0006457	Protein folding	Ptdins pathway
GO:0004722	Protein serine/threonine phosphatase	Pyruvate metabolism
GO:0004725	Protein tyrosine phosphatase activity	Rarrxr pathway
GO:0016567	Protein ubiquitination	RNA polymerase
GO:0015992	Proton transport	Stress pathway
GO:0008217	Regulation of blood pressure	Tid pathway
GO:0042127	Regulation of cell proliferation	Wnt pathway
GO:0006446	Regulation of translational initiation	
GO:0006950	Response to stress	
GO:0005840	Ribosome	
GO:0003723	RNA binding	
GO:0008380	RNA splicing	
GO:0006814	Sodium ion transport	
GO:0030528	Transcription regulator activity	
GO:0003743	Translation initiation factor activity	
GO:0006512	Ubiquitin cycle	
GO:0000151	Ubiquitin ligase complex	
GO:0006511	Ubiquitin-dependent protein catabolism	
GO:0004842	Ubiquitin-protein ligase activity	
GO:0030018	Z disc	
GO:0008270	Zinc ion binding	

GSEA, Gene set enrichment analysis; SAFE, Significance Analysis of Functional Expression; LVH, left ventricular hypertrophy; GO, gene ontology.

TABLE E4. Significant gene ontologies and GSEA categories identified by means of SAFE analysis in both LVH progression and regression

GO:0005516	Calmodulin binding
GO:0003677	DNA binding
GO:0005874	Microtubule
GO:0005762	Mitochondrial large ribosomal subunit
GO:0003704	Specific RNA polymerase II transcription factor activity
GO:0004221	Ubiquitin thiolesterase activity

GSEA, Gene set enrichment analysis; *SAFE*, Significance Analysis of Functional Expression; *LVH*, left ventricular hypertrophy; *GO*, gene ontology.