Wnt Signaling Is Critical for Maladaptive Cardiac Hypertrophy and Accelerates Myocardial Remodeling


Abstract—The evolutionary conserved Wnt signaling pathway regulates cardiogenesis. However, members of the Wnt pathway are also expressed in the adult heart. Although Wnt-signaling is quiescent under normal conditions, we noticed activation on pathological stress of the heart, such as chronic afterload increase. To examine the role of Wnt signaling on the postnatal heart, we modified the expression and function of the Wnt regulator dishevelled 1 (Dvl-1) both in transgenic mice with cardiac-specific overexpression of Dvl-1 (Dvl-1-Tg) and in cultured cardiac myocytes. Dvl-1-Tg mice (3 months) had severe cardiac hypertrophy (heart weight/body weight ratio: 5.2±0.3 mg/g wild-type [WT] versus 6.4±0.7 mg/g Dvl-1-Tg; P<0.01), an increase in cardiomyocyte size (86% increase in Dvl-1-Tg compared with WT; P<0.01) and marked raise of atrial natriuretic factor expression (12-fold increase versus WT; P<0.01). Hypertrophy was associated with left ventricular dilatation in Dvl-1-Tg and a reduction of ejection fraction (4.4±0.1 mm versus 5.5±0.2 mm, 80±2% and 43±4% in WT versus Dvl-1-Tg, respectively; P<0.01). Transgenic animals died prematurely before 6 months of age. Both canonical as well as noncanonical Wnt signaling branches were activated in the Dvl-1-Tg animals. Small interfering RNA–mediated depletion of Dvl-1 was used to further characterize the role of Dvl-1 in cardiac myocytes. Whereas baseline parameters were unaltered, β-adrenergic hypertrophic response was abrogated in Dvl-1 knockdown cardiac myocytes, indicating a mandatory role in β-adrenergic stimulation. Therefore, activation of Wnt signaling is sufficient and critical for the induction of myocardial hypertrophy and cardiomyopathy. (Hypertension. 2010;55:939-945.)

Key Words: Wnt signaling ▶ cardiac hypertrophy ▶ experimental heart failure

A fter conditions of increased wall stress, the myocardium may adopt by a process called remodeling. Left ventricular remodeling is considered to be a maladaptive process characterized by myocyte hypertrophy, an increase in myocardial fibrosis, and left ventricular dilatation. Remodeling may induce clinically overt heart failure and contributes to increased mortality after myocardial infarction. Other than standard heart failure medications, there are no treatment options available targeted to maladaptive remodeling. On pathological stress, the heart reactivates a number of signaling pathways, which traditionally were thought to be operational primarily in the developing organism. The Wnt pathway is an evolutionary conserved signaling mechanism with a critical function in tumor growth and cardiogenesis. However, members of the Wnt signaling pathway are also expressed in the adult heart. Although Wnt-signaling is quiescent under normal conditions, we noticed activation on pathological stress of the heart, such as chronic afterload increase. To examine the role of Wnt signaling on the postnatal heart, we modified the expression and function of the Wnt regulator dishevelled 1 (Dvl-1) both in transgenic mice with cardiac-specific overexpression of Dvl-1 (Dvl-1-Tg) and in cultured cardiac myocytes. Dvl-1-Tg mice (3 months) had severe cardiac hypertrophy (heart weight/body weight ratio: 5.2±0.3 mg/g wild-type [WT] versus 6.4±0.7 mg/g Dvl-1-Tg; P<0.01), an increase in cardiomyocyte size (86% increase in Dvl-1-Tg compared with WT; P<0.01) and marked raise of atrial natriuretic factor expression (12-fold increase versus WT; P<0.01). Hypertrophy was associated with left ventricular dilatation in Dvl-1-Tg and a reduction of ejection fraction (4.4±0.1 mm versus 5.5±0.2 mm, 80±2% and 43±4% in WT versus Dvl-1-Tg, respectively; P<0.01). Transgenic animals died prematurely before 6 months of age. Both canonical as well as noncanonical Wnt signaling branches were activated in the Dvl-1-Tg animals. Small interfering RNA–mediated depletion of Dvl-1 was used to further characterize the role of Dvl-1 in cardiac myocytes. Whereas baseline parameters were unaltered, β-adrenergic hypertrophic response was abrogated in Dvl-1 knockdown cardiac myocytes, indicating a mandatory role in β-adrenergic stimulation. Therefore, activation of Wnt signaling is sufficient and critical for the induction of myocardial hypertrophy and cardiomyopathy. (Hypertension. 2010;55:939-945.)

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From the Departments of Cardiology (P.M., M.H., S.B., M.R.S., C.S.W., D.W., J.R., A.B., H.A.K., S.E.H.) and Anesthesiology (A.A.), University of Heidelberg, Heidelberg, Germany.

P.M. and M.H. contributed equally to this article.

Correspondence to Stefan E. Hardt, Department of Cardiology, Angiology, and Pulmology, University of Heidelberg, Im Neuenheimer Feld 410, 69120 Heidelberg, Germany. E-mail stefan.hardt@med.uni-heidelberg.de

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Materials and Methods
The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health and was approved by the local animal ethics review board (approval No. 40631), as well as by the Regierungspräsidium Karlsruhe (project No. 35-9185.81/G-131/06 for the rat and 35-9185.81/G81-08 for the mouse model). Isolation and culture of cardiac myocytes, small interfering RNA (siRNA) transfection, immunoblot analyses, quantitative real-time PCR, and determination of myocyte surface area were performed as described previously. For descriptions about specifics in the methods used in this study and the generation of transgenic mice please refer to the online Data Supplement at http://hyper.ahajournals.org.

Aortic Banding and Heart Failure Model
Aortic banding was induced in rats by clipping the ascending aorta just above the coronary arteries. Banded animals develop compensated cardiac hypertrophy without heart failure after 4 weeks. Heart failure was induced in a swine model of atrial fibrillation (AF) induced by pacemaker stimulation. The model and details about pacemaker implantation and further experimental settings have been described previously.

Histopathology
Hematoxylin and eosin, Masson trichrome, and TUNEL staining were performed according to standard protocols. Please see the online Data Supplement for a complete description of methods related to histopathology used for these studies.

Echocardiography and Pressure-Volume Loop Analysis
Echocardiography and invasive hemodynamics with conductance catheters were done under light anesthesia with isoflurane as outlined in detail in the online Data Supplement.

Statistical Analysis
Data are reported as the mean±SEM. Difference between the groups was compared by 2-tailed Student t test or by multiple ANOVA, when applicable. A post hoc test was performed by the method of Tukey. Significance was accepted at the \( P<0.05 \) level.

Results

Pressure Overload and Chronic Heart Failure Reactivate Myocardial Wnt Signaling
In aortic stenosis tissues in the stable phase of cardiac hypertrophy 4 weeks after aortic banding an increased protein expression of Dvl-1 was observed (Figure 1A). Furthermore, in a porcine model of persistent AF with chronic heart failure, Western blot analysis of left ventricular tissues revealed an overexpression of Dvl-1 (Figure 1B). This prompted us to further study the role of Dvl-1 in the postnatal heart.

Activation of Wnt Signaling Leads to Cardiomyopathy and Premature Death
To study the role of Wnt signaling in the postnatal heart, we generated mice with cardiac-specific overexpression of Dvl-1 under the control of an α-myosin heavy chain promoter (Figures 1C and S1). All of the experiments were performed in animals 3 months of age unless stated otherwise.

Severe cardiomyopathy in Dvl-1 transgenic (Tg) mice was evident from gross pathology of transgenic hearts (Figure 1D). Premature death occurred in Dvl-1-Tg mice before the age of 6 months (Figure 1E). Postmortem analysis of these animals

![Image](http://hyper.ahajournals.org/)

Figure 1. A, Upregulation of Dvl-1 in a rat model of chronic aortic stenosis. Representative Western blot showing an increase in the expression of Dvl-1 in left ventricular tissues from animals with aortic stenosis compared with sham animals; \( n=5 \) in each group. B, Upregulation of Dvl-1 in a porcine model of AF with severe heart failure. Western blot demonstrating an increase in the expression of Dvl-1 in left ventricular tissues from animals with severe chronic heart failure compared with sham animals; \( n=4 \) in each group. Coomassie staining of polyvinylidene fluoride membrane shows equal loading of sodium dodecyl sulfate gel. C, Confirmation of the overexpressions of Dvl-1 at the protein level by immunoblot analysis. D, Dvl-1-Tg mice exhibited severe cardiomyopathy and grossly dilated hearts when compared with WT animals. The phenotype was prevalent at 3 months of age. E, Survival curve depicting the trend of mortality of Dvl-1-Tg mice in weeks; \( n=21 \).
revealed hypertrophic hearts and signs of pulmonary congestion indicating severe cardiomyopathy. Echocardiographic analysis of animals aged 3 months showed an increase in end-diastolic diameter (5.5 ± 0.2 versus 4.4 ± 0.1 mm) and end-systolic diameter (4.8 ± 0.2 versus 2.6 ± 0.1 mm) in Dvl-1-Tg mice as compared with wild-type (WT) animals leading to a marked reduction in left ventricular ejection fraction in Dvl-1-Tg mice (43 ± 4% versus 80 ± 2%; *P < 0.01; Figure 2A, online Data Supplement movies I and II).

Contractility of the left ventricle was further assessed by pressure-volume loop analysis. The increase in end-diastolic and end-systolic volumes was associated with a decrease in the maximal rise of left ventricular pressure (dp/dt maximum: 8742 ± 664 versus 5022 ± 322 mm Hg/s), indicating impairment of contractility in Dvl-1-Tg mice (Figure 2B and 2C). Reduction in dp/dt minimum levels (−8511 ± 402 versus 4785 ± 311 mm Hg/s) and the increase in the time constant of isovolumic relaxation (Tau-Glantz) levels demonstrate diastolic dysfunction in the Dvl-1-Tg mice (7.3 ± 0.3 versus 15.08 ± 2.0 m/s; *P < 0.05; **P < 0.01 vs control). Taken together, echocardiography and invasive hemodynamics consistently demonstrated a significant impairment of myocardial function in Dvl-1-Tg mice.

**Dvl-1 Is Sufficient and Critical to Induce Cardiac Hypertrophy**

Myocardial dysfunction in Dvl-1-Tg mice was associated with myocardial hypertrophy and morphological characteristics of adverse remodeling. Heart weight/body weight ratio (in milligrams per gram) of fresh tissue (6.2 ± 0.5 versus 4.8 ± 0.2 mg/g; *P < 0.01) and heart weight/tibia length ratio (in milligrams per milliliter) were significantly higher in Dvl-1-Tg mice (10.36 ± 0.8 versus 7.1 ± 0.2 mg/mm; Figure 3A). Furthermore, increased cardiomyocyte size in Dvl-1-Tg mice shown by hematoxylin and eosin staining (Figure 3A and 3B) and increased fibrosis with extensive collagen deposition in
the left ventricle as observed by Masson trichrome staining were observed (Figure 3C). Atrial natriuretic factor, a molecular marker of cardiac hypertrophy, showed a 12-fold increase on mRNA level in Dvl-1-Tg mice when compared with WT mice (Figure 3D). Heart failure in Dvl-1-Tg was accompanied by a significant increase in apoptosis in Dvl-1-Tg mice (Figure S2). We further examined the effect of siRNA-mediated depletion of Dvl-1 in cardiac myocytes.

Figure 3. A, Histopathologic analysis revealed cardiac hypertrophy. Increase in heart weight:body weight ratio was noted in Dvl-1-Tg mice relative to WT mice. n=24 in each group. Increase in heart weight:tibia length ratio was noticed in Dvl-1-Tg mice relative to WT; n=11 WT and n=22 Dvl-1-Tg. Increased cardiac myocyte size in Dvl-1-Tg mice as assessed by hematoxylin and eosin staining. Bar graph showing myocyte size relative to WT animals; n=7 in each group. Data are mean±SEM; *P<0.05, **P<0.01 vs control. B, Representative microphotographs of hematoxylin and eosin–stained myocardial tissue sections. C, Extensive fibrosis is observed in the left ventricle of the Dvl-1-Tg mice by Masson trichrome staining; n=6. D, Quantitative PCR analysis revealed an increase in expression of hypertrophy marker atrial natriuretic factor (ANF); n=6 in each group. Data are mean±SEM; **P<0.01 vs control.

Figure 4. siRNA-mediated depletion of Dvl-1 does not lead to baseline alterations in unstimulated cells. However, in Dvl-1–depleted cardiac myocytes, isoproterenol treatment failed to induce a hypertrophic response. A, Western blot demonstrating successful siRNA-mediated knockdown of Dvl-1 by ~80%. B, Representative pictures depicting the effect of siRNA-mediated depletion of Dvl-1 in cardiac myocytes (left). C, Relative myocyte size is given in bar graphs (right). Data are mean±SEM; *P<0.05 vs control.
Although no baseline alterations in unstimulated cells were observed when Dvl-1 was depleted, isoproterenol challenge failed to induce a hypertrophic response in cardiac myocytes under these conditions, indicating that Dvl-1 is necessary for β-adrenergic cardiac hypertrophy (Figure 4).

Involvement of Canonical and Noncanonical Wnt Signaling Pathways in Cardiac Hypertrophy

Overexpression of Dvl-1 leads to the accumulation of β-catenin, the key molecule of canonical Wnt signaling (Figures 5A and S3). β-Catenin further translocates into the nucleus and interacts with the lymphoid enhancer factor/T cell factor family of transcription factors. β-Catenin thereby induces the transcription of its target genes. In line, the proto-oncogenes cyclin D1 and c-Myc were upregulated in Dvl-1-Tg mice (Figures 5A, S3, and S4). Moreover, mRNA expression of axin 2, a prominent indicator of activation of the canonical Wnt signaling pathway, was increased (Figure S5). In addition, noncanonical pathways were found to be activated after Dvl-1–induced activation of Wnt signaling. Noncanonical Wnt signaling divides into 3 major branches mediated through c-Jun N-terminal kinases (JNKs), protein kinase C (PKC), and Ca²⁺/calmodulin-dependent protein kinase II (CAMKII). In Dvl-1-Tg mice, JNK signaling was enhanced. Furthermore, CAMKII and PKC signalings were activated (Figures 5B and S6). Phosphorylation of CAMKII was associated with increased phosphorylation of histone deacetylase 4, a class II histone deacetylase that has been associated with cardiac hypertrophy (Figures 5C and S7).

Because PKC and CAMKII are known to be activated during the course of cardiac hypertrophy, we further investigated whether activation of these molecules was the cause or consequence of the cardiomyopathic phenotype of Dvl-1-Tg animals. Activation of these pathways could be found in 6-week–old animals in which the cardiac phenotype was still identical to WT animals (Figure S8), indicating a causal link of noncanonical Wnt signaling to cardiomyopathy.

**Discussion**

Wnt signaling is highly conserved among species and plays a major role in development and tumorigenesis. In this study we identified Wnt signaling to be central to myocardial hypertrophy and function. Our data demonstrated activation of Wnt signaling in the chronic phase of pressure-overload hypertrophy in rats, as well as in a porcine model of AF with chronic heart failure.

Molecules and pathways that are differentially regulated in embryonic and fully developed myocardium may be re-

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**Figure 5.** Activation of canonical and noncanonical pathways in Dvl-1-Tg mice. A, Representative Western blots showing β-catenin, cyclin D1, and c-Myc of the canonical pathway. B, Representative Western blots showing JNK1, phospho-stress-activated protein kinase/JNK (Thr183/Tyr185), CaMKII, phospho-CaMKII (Thr286), PKCδ, phospho-PKCδ (Thr 505), PKCα, and phospho-PKCα/β (Thr 638/641) of the noncanonical pathway. C, Representative Western blot showing histone deacetylase (HDAC) 4 and phospho-HDAC4 (Ser 632).
expressed under conditions of inappropriate stress or pathological stimuli. The molecules involved in Wnt signaling share these properties. Wnt signaling is critical for proper myocardial differentiation but silenced in normal fully differentiated myocardium. Our finding that Wnt signaling is activated in the overloaded heart suggests not only a specific role in the disease process but may also provide novel opportunities for treatment of myocardial hypertrophy and heart failure. This notion is further supported by our finding that depletion of Dvl-1 did not lead to alterations of baseline morphology, which indicates that Dvl-1 is not centrally involved in normal cardiac growth but primarily appears to mediate pathological hypertrophy, thus serving as an option to specifically target maladaptive cardiac hypertrophy.

The function of Wnt signaling in the postnatal heart has not been systematically examined thus far. Few studies have investigated the role of β-catenin–dependent signaling. In this context, Haq et al. demonstrated that activation of β-catenin is not only sufficient but also necessary to induce cardiomyocyte hypertrophy. Interestingly, the data demonstrate that stabilization of β-catenin apparently can occur through stimulation of G protein–coupled receptors, suggesting that β-catenin participates here in a Wnt independent manner, which has also been demonstrated by Liu et al. This finding is supported by results from this study demonstrating that β-adrenergic stimulation, which activates Gs-dependent signaling, depends on the presence of Dvl-1 to induce cardiomyocyte hypertrophy.

The importance of β-catenin–dependent signaling for the development of cardiac hypertrophy has also been confirmed in vivo using mice with conditional cardiac-specific knockout of β-catenin. In line with these previous findings, Qu et al demonstrated that cardiac-specific haploinsufficiency for β-catenin attenuates pressure-overload–induced cardiac hypertrophy. In another study by Hahn et al., overexpression of a constitutively active form of β-catenin led to spontaneous hypertrophy of cardiomyocytes in culture. van de Schans et al. reported that mice lacking the Dvl-1 gene showed attenuated hypertrophic response to pressure overload, which was related to altered activity of glycogen synthase kinase 3β and AKT. Of note in this model, where deletion of Dvl-1 was not restricted to the heart, an increase of β-catenin was inhibited after aortic banding, whereas downstream targets of β-catenin were not regulated. Conflicting with these previous results, Baurand et al. reported spontaneous cardiac hypertrophy in β-catenin–depleted mice and attenuation of angiotensin II–induced cardiac hypertrophy in mice with stabilized β-catenin. These findings, however, were on the basis of analysis of cardiomyocyte size and atrial natriuretic factor expression, whereas total heart weight was not measured. Although cyclin D1 is a known target of β-catenin, Haq et al. found that this gene is not activated in cardiomyocytes in vitro when β-catenin is overexpressed. However, in our mouse model, a significant upregulation of cyclin D1 was observed, which suggests the activation of the lymphoid enhancer factor/T cell factor family of target genes in the heart in vivo.

By contrast to canonical Wnt signaling, the role of noncanonical Wnt signaling in postnatal hearts and specifically its potential significance in the context of myocardial disorders have not been investigated thus far. This is surprising given that molecules such as PKC, CAMKII, and Rho are not only members of noncanonical Wnt signaling but are also involved in myocardial remodeling. In this context, translocation modification has confirmed that PKCδ is a critical mediator of postschismic cardiomyocyte necrosis and contractile dysfunction in mice, rats, and pigs and that PKCα regulates cardiac contractility and mediates the development of heart failure. Recently, Backs et al. showed that CaMKIIδ-null mice have attenuated response to pressure overload.

In our model we demonstrate for the first time that both canonical and noncanonical Wnt pathways are activated in Dvl-1-Tg mice leading to a severe cardiomyopathy phenotype. Key proteins of the canonical (β-catenin, cyclin D1, c-Myc, and axin 2), as well of the noncanonical, Wnt pathway (JNK, PKC, and CAMKII) are upregulated, suggesting the involvement of both Wnt branches in cardiac hypertrophy (Figure 6). The absence of activated Wnt signaling prevents hypertrophic cardiomyocyte growth.

**Perspectives**

Our data provide compelling evidence that all of the branches of Wnt signaling are involved in cardiac hypertrophy and cardiomyopathy. Inhibition of Dvl-1–dependent Wnt signaling could serve as a lead target for the treatment of cardiac hypertrophy.

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Disclosures

None.

References


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Supplementary materials and methods

siRNA transfection
siRNAs used were 5´-AGAUCACCAUUGCCAAUGC-3´ for Dvl-1 and 5´-AGGUAGUGUAAUCGCCUUGTT-3´ for non-specific siRNA. Myocytes transfected with non-specific siRNA were used as controls. siRNAs were synthesized by MWG (Ebersberg, Germany).

Immunoblot analyses
Antibodies used were anti-Dvl-1 (Santa Cruz), anti-β-catenin (Santa Cruz), anti-Cyclin-D1 (Santa Cruz), anti c-Myc (Cell Signaling), anti JNK1 (Santa Cruz), anti phospho SAPK/JNK (Thr183/185) (Cell signaling), anti-CaMKII (BD Transduction Laboratories), anti-ActiveCaMKII(Thr286) (Promega), PKC δ (Cell Signaling), Phospho PKCδ (Thr 505) (Cell Signaling), PKCα (Cell Signaling), Phospho PKC α/βII (Thr 638/641) (Cell Signaling),anti HDAC4 (Santa Cruz), ant Phospho HDAC4 (Cell Signaling) and anti-α-actin (Sigma-Aldrich). Anti-rabbit IgG and anti-mouse IgG horseradish peroxidase-conjugated antibodies (Cell Signaling Technology) were used as secondary antibodies. Bands were quantified by densitometry using the Image J programe.

Quantitative real-time PCR
Following primers and probes were used. For HPRT 5´-gtcaaggggacataaaag-3´ and 5´-tgcatgtttaccaagtaca-3´, probe #22; ANF 5´-caacacagatggtgag-3´ and 5´-tcttcctcatcttcttc-3´, probe #25; c-myc 5´-ccctcttcggttc-3´ and 5´-tctttctctctttcttc-3´, probe #77 and for Axin-2 5´-gagagtgagcggcagagc-3´ and 5´-cggctgactcgtctctc-3´, probe #96

Transgenic mouse model
The murine Dvl-1 cDNA with an amino-terminal HA epitope tag was cloned into an α-MHC promoter construct. Microinjection was performed into C57BL/6 mouse embryos. PCR and Southern blotting was used to screen for founders and transgenic mice. Four transgenic founders were born alive. One of the founder animals was dead before starting mating. The line with highest overexpression was chosen as working line.

Histopathology
Mice were euthanized and left ventricles were isolated and were further fixed in formalin overnight, paraffin embedded, and processed for sectioning. 0.5µM sections were made and stained with haematoxylin and eosin (H&E) to determine the myocyte size and with Masson’s trichrome to detect collagen deposition. Evidence of fibrosis was evaluated in a blind manner by an investigator who used light microscopy, according to a 6-tier scoring system: grade 0, no fibrosis; grade 1, cardiac fibrosis in up to 5% of the cardiac sections; grade 2, 6% to 10%; grade 3, 11% to 30%; grade 4, 31 to 50% and grade 5, >50%. The score from the investigator’s reading was taken for statistical analysis with a nonparametric test. Apoptosis was measured by TUNEL assay in the tissue sections. TUNEL assays on tissue sections were done according to the manufacturer’s instructions (Roche diagnostics). Sections were briefly rehydrated, treated for 8 min with PBS containing 0.1% Triton and 0.1% Sodium citrate and incubated with terminal deoxynucleotidyl transferase in the presence of Fluoresceein-labeled dUTP (60 min at 37°C) and counterstained with Hoechst 33258 (3.5 µg/ml). The frequency of TUNEL positive cells was quantified by blinded investigators.

Echocardiography
For echocardiography mice were anesthetized by isofluorane inhalation (~2% isofluorane).M-mode measurements of left ventricular dimensions were takeb3 readings per mouse. LV percent fractional shortening (LVFS) is calculated as follows: LVFS=[(EDD-ESD)/EDD]³ x100, where EDD indicates end-diastolic and ESD indicates end-systolic diameter. The observer was unaware of the genotype.
Pressure-volume loop analysis
Briefly this method is based on measuring the time-varying electrical conductance signal of two segments of blood in the LV from which the total LV volume is calculated. The raw conductance volumes were corrected for parallel conductance by the hypertonic saline dilution method. Data were digitized with a sampling rate of 1000Hz and recorded on a PC using software package (Chart, AD instruments). Subsequent analysis of pressure-volume loops was done by using PVAN software (Millar Instruments Inc).
Supplementary Movies I and II:

**Movie I**

[Image of echocardiogram of WT mouse]

**Movie II**

[Image of echocardiogram of Dvl-Tg mouse]

**Movie I and II:** Movies displaying two-dimensional echocardiograms of WT and Dvl-Tg-mice. Normal left ventricular diameters and function are present in WT (movie I), while left ventricular dilatation and depressed LV-function is evident in Dvl-1-Tg (movie II).
Fig. S1: Generation of Dvl-1 overexpressed transgenic mice. Dishevelled-1 cDNA was cloned into the multiple cloning site of the Vector pcDNA3.1 under the control of cardiac specific α-MHC promoter.

Fig. S2: Increase in Apoptosis in the LV of Dvl-1-Tg mice compared to WT mice. Frequency of apoptosis in the left ventricle, as determined by TUNEL assay. Bar graph summarizing frequency of apoptosis in the LV.; N=6 in each group. Data are means ± SEM; *p<0.01 vs. control.
Supplementary Figure S3

**Fig. S3:** Activation of the canonical Wnt signaling pathway. In Dvl-1-Tg animals protein levels of β-catenin and target genes c-myc and cyclin-D1 were upregulated. N=6 in each group. Data are means ± SEM; **p<0.01 vs. control.

Supplementary Figure S4

**Fig. S4:** Quantitative PCR analysis revealed a 1.8 fold increase in c-myc in the LV of Dvl-1-Tg mice compared to WT mice. Bar graph shows mean ± SE of 5 animals in each group (*p<0.01 vs. WT). All measurements were normalized to HPRT expression.
Fig. S5: Quantitative PCR analysis revealed a 2 fold increase in Axin-2 in the LV of Dvl-1-Tg mice compared to WT mice. Bar graph shows mean ± SE of 5 animals in each group (*p<0.01 vs. WT). All measurements were normalized to HPRT expression.
Supplementary Figure S6

Fig. S6: Activation of non-canonical Wnt signaling pathway. In Dvl-1-Tg animals all three branches of the non-canonical Wnt signaling, JNK- CamKII- and PKC-signaling pathway were activated. N=6 in each group. Data are means ± SEM; * p<0.05, **p<0.01 vs. control.
Supplementary Figure S7

**Fig. S7:** Phosphorylation of HDAC-4 in Dvl-1-Tg animals compared to WT. N=6 in each group. Data are means ± SEM; * p<0.05 vs. control.
Supplementary Figure S8

**Fig. S8:** Activation of non-canonical pathway in 6 weeks old Dvl-Tg mice. Representative western blots showing Phospho-CaMKII (Thr286) and Phospho-SAPK/JNK (Thr183/Tyr185). Data are means ± SE; *p<0.01 vs.control.