Dapper-1 Induces Myocardial Remodeling Through Activation of Canonical Wnt Signaling in Cardiomyocytes

Marco Hagenmueller, Johannes H. Riffel, Elmar Bernhold, Jingjing Fan, Min Zhang, Marco Ochs, Herbert Steinbeisser, Hugo A. Katus, Stefan E. Hardt

Abstract—Heart failure has an increasing contribution to cardiovascular disease burden and is governed by the myocardial remodeling process. The contribution of Wnt signaling to cardiac remodeling has recently drawn significant attention. Here, we report that upregulation of Dapper-1 in a transgenic mouse model activates the canonical/β-catenin–dependent Wnt pathway through dishevelled-2. These mice exhibited increased heart weight/tibia length ratio, myocyte cross-sectional area, and upregulation of hypertrophic marker genes compared with wild-type mice. Furthermore, impairment of left ventricular systolic and diastolic function was observed in all indicating features of myocardial remodeling. Depletion of Dapper-1 and dishevelled-2 in cardiomyocytes demonstrated that Dapper-1 functions upstream of dishevelled-2 and that activity of both Dapper-1 and dishevelled-2 is essential for activating canonical Wnt signaling. Moreover, Dapper-1 depletion alleviated Wnt3a- and phenylephrine-induced cardiomyocyte hypertrophy. These observations provide evidence that Dapper-1–mediated activation of canonical Wnt signaling is necessary and sufficient to induce cardiomyocyte hypertrophy. Inhibition of this pathway may thus serve as a novel therapeutic strategy for alleviating cardiac hypertrophy. (Hypertension. 2013;61:1177-1183.) ● Online Data Supplement

Key Words: cardiac remodeling, ventricular ▪ transgenic mice ▪ Wnt signaling pathway

Improvement of medical treatment algorithms and technologies resulted in increased survival of myocardial infarction throughout the past years. Concomitantly, cardiovascular morbidity attributable to heart failure and its underlying myocardial remodeling processes is spreading in industrialized countries. Significant improvement in treatment strategies for these patients is required to close the gap of these unmet needs.

The Wnt signaling pathway is evolutionarily conserved throughout the metazoa and controls various developmental processes, including early cardiogenesis. The Wnt cascade is inactive in adult hearts; however, recent studies indicate that this pathway is involved in myocardial remodeling after pathological injury. This is supported by our recent study in which cardiac-specific overexpression of dishevelled (Dvl1) in mice activates the Wnt pathway and induces severe cardiac remodeling.

Dvl proteins are fundamental molecules to regulate Wnt activity. They exist in 3 isoforms (Dvl1–Dvl3) with partly redundant functions. In canonical Wnt signaling, Wnt ligands bind to frizzled receptors and recruit Dvl to the plasma membrane. In turn, Dvl mediates the stabilization and accumulation of β-catenin in the cytoplasm and its translocation into the nucleus, where it interacts with transcription factors of the T-cell factor/lymphoid enhancer factor (TCF/LEF) family to induce the expression of specific targets, including c-myc, cyclin D1, and peroxisome proliferator-activated receptor-δ.

Among other members of the Wnt pathway, Dapper-1 (Dpr1, also called Dact1 or Frodo) interacts with Dvl proteins in a tissue- and context-dependent manner. Although, so far in general, Dpr1 is considered as a negative Wnt regulator, its substantial contribution to Wnt signaling is not unambiguous.

To date, the role of Dpr1 in cardiac tissue and isolated cardiomyocytes remains unexplored. We observed strong upregulation of Dpr1 in the remote area 1 week after myocardial infarction and in isolated cardiomyocytes cultured in hypoxia conditions, leading us to further examine the role of Dpr1 for myocardial function and in Wnt signal transduction. We present evidence that Dpr1 is a positive regulator of canonical Wnt signaling in myocardial tissue and in cultured cardiomyocytes, and show furthermore that Dpr1-mediated activation of the canonical Wnt pathway induces cardiac remodeling and impairs left ventricular (LV) function.

Materials and Methods

Detailed description of all materials and methods used are provided in the online-only Data Supplement.

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Robust Overexpression of Dpr1 in a Rat Myocardial Infarction Model

We observed robust overexpression of Dpr1 protein and mRNA in the remote area of rat myocardial infarction tissue and in isolated cardiomyocytes, cultured under hypoxia conditions (Figure 1A and 1B, Figure S1A–S1D in the online-only Data Supplement), suggesting involvement of Dpr1 in early cardiac remodeling. In addition, Dpr1 accumulation in the cytoplasm and increased nuclear localization in hypoxia cells were determined (Figure 1C). This prompted us to elucidate the function of Dpr1 in the heart. Therefore, we genetically engineered transgenic mice (Dpr1-tg) with cardiac-specific overexpression of Dpr1 under the control of an α–myosin heavy chain promoter. Upregulation of Dpr1 in cardiac tissue was confirmed by immunoblotting (Figure S1E). Animals were characterized at 3 months of age.

Dpr1 Induces Cardiac Hypertrophy and Impairs LV Function

Dpr1-tg mice exhibited a higher heart weight/tibia length ratio and increased left and right ventricular weight/tibia length ratio than wild-type (WT) animals (7.6±0.2 mg/mm versus 7.0±0.2 mg/mm, 5.6±0.1 mg/mm versus 5.2±0.1 mg/mm, 1.3±0.09 mg/mm versus 1.1±0.05 mg/mm, respectively; P<0.05; Figure 2A–2C). Cardiomyocyte cross-sectional area was increased in mice overexpressing Dpr1 (+21±2% versus WT animals; P<0.05; Figure 2D and 2E). In line with these findings, upregulation of prominent hypertrophy marker genes atrial natriuretic factor, brain natriuretic peptide, and β–myosin heavy chain was observed (Figure 2F). Baseline echocardiography revealed no significant difference between Dpr1-tg mice and WT animals in ejection fraction (Figure 2G), end-systolic, and end-diastolic diameter (data not shown). However, cardiac hypertrophy was associated with impairment of LV function. Pressure–volume loop analysis revealed a decrease in the maximal rise of LV pressure (dp/dt max; 10 878±2669 mm Hg/s in WT versus 8498±2044 mm Hg/s in Dpr1-tg mice; P<0.005; Figure 2H), indicating impaired contractility in Dpr1-tg mice. The reduction in dp/dt min levels (−8920±435 mm Hg/s in WT versus −7529±451 mm Hg/s in Dpr1-tg mice; P<0.01; Figure 2I) and the simultaneous increase in the time constant of isovolumic relaxation (10.24±0.6 m/s in WT mice versus 12.2±0.3 m/s in Dpr1-tg mice; P<0.01; Figure 2J) demonstrate signs of diastolic dysfunction compatible with the myocardial hypertrophy observed.

Dpr1 Activates Canonical Wnt Signaling Pathway in the Murine Heart

Dpr1-tg mice had increased Dvl2 protein levels and mRNA transcripts, whereas expression of Dvl1 and Dvl3 isoforms remained unaltered (Figure 3A, Figure S2A and S2B). In addition, we observed enhanced Dvl2 phosphorylation (Figure 3A), indicating higher Dvl2 activity. Given the redundancy of Dvl proteins, we asked whether this was sufficient to activate canonical Wnt signaling and found increased total- and activated β-catenin amounts, but reduction of β-catenin (pS33/37, pT41; Figure 3B and Figure S2C). Phosphorylation of β-catenin primes this protein for degradation. Immunostaining of LV tissue slides revealed β-catenin accumulation in the cytoplasm and its translocation into the nucleus (Figure 3C). Steady-state levels of β-catenin mRNA transcripts suggest post-translational stabilization (Figure S2D). Stabilized β-catenin was accompanied by transcription of Wnt/β-catenin targets c-myc, cyclin D1, peroxisome proliferator-activated receptor-β, and myoD, all indicating activation of canonical Wnt signaling (Figure 3D).

Dpr1 Is Both Necessary and Sufficient to Activate Canonical Wnt Signaling in Cardiomyocytes

We further mapped the molecular mechanism by which Dpr1 impacts canonical Wnt signaling in cultured cardiomyocytes by siRNA-mediated Dpr1 depletion. Consistent with our observations in Dpr1-tg mice, we detected decreased Dvl2 protein abundance but unaltered expression of Dvl1 and

Figure 1. Robust overexpression of Dapper-1 (Dpr1) in a rat myocardial infarction (MI) model. A, Western blot showing upregulation of Dpr1 in murine MI tissue obtained 1 week after surgery. B, Western blot showing increased Dpr1 protein content in cardiomyocytes after hypoxia. Coomassie staining was used as loading control. C, Fluorescence image of cardiomyocytes exposed to hypoxia for 24 hours. Dpr1 (red) accumulates in the cytoplasm and translocates into the nucleus (blue).
Dvl2 mRNA copies were not reduced, suggesting a mechanism on levels after transcription (Figure S3B). Inhibition of lysosome acidification with NH4Cl stabilized Dvl2 proteins in Dpr1-depleted cells, indicating that downregulation of Dvl2 is a result of lysosomal degradation (Figure 4B and Figure S3C). Downstream of Dvl2, these cells displayed reduced total- and active β-catenin protein amounts, as well as inhibition of c-myc protein expression (Figure 4A and Figure S3B). Copy number of β-catenin mRNA was stable, suggesting that decreased protein content is a result of post-translational reduction (Figure S3E).

Wnt3a-conditioned medium specifically activates β-catenin–dependent Wnt signaling. Cellular distribution of Dpr1 on Wnt3a-stabilized Dvl2 proteins in Dpr1-depleted cells, indicating that downregulation of Dvl2 is a result of lysosomal degradation (Figure 4B and Figure S3C). Downstream of Dvl2, these cells displayed reduced total- and active β-catenin protein amounts, as well as inhibition of c-myc protein expression (Figure 4A and Figure S3B). Copy number of β-catenin mRNA was stable, suggesting that decreased protein content is a result of post-translational reduction (Figure S3E).

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Dpr1-Induced Activation of Canonical Wnt Signaling Is Dvl2 Dependent

Correlation between the protein abundances of Dpr1 and Dvl2 was observed in Dpr1-depleted cardiomyocytes, and Wnt3a stimulation failed to enhance TOPFlash reporter activation (Figure 4E); Conversely, Dpr1 overexpression enhances both β-catenin and TCF/LEF reporter activity under basal conditions and synergized with the effect of Wnt3a (Figure 5F and 5G, Figure S3H).
In addition, Dpr1-induced TOPflash reporter activation was completely inhibited in cells lacking Dvl2 (Figure 5H), demonstrating that Dvl2 is a required cofactor for transmitting canonical Wnt signals downstream of Dpr1.

Wnt3a- and PE-Induced Hypertrophy Is Inhibited in Dpr1-Depleted Cardiomyocytes

Myocyte surface area increased in response to Wnt3a stimulation (Figure S4A) as did protein synthesis (Figure S4B), indicating that stimulation of the canonical Wnt pathway alone is sufficient to induce a hypertrophic response. In a complementary approach, we found that Dpr1 depletion and concomitant inhibition of the canonical Wnt pathway rescued Wnt3a-induced cardiomyocyte hypertrophy. The cardiomyocyte surface area was smaller in Dpr1 knockdown cells (Figure S4A), and Wnt3a-induced protein synthesis was completely blocked, indicating that Dpr1 is required for Wnt3a-induced hypertrophy (Figure S4B). In addition, Dpr1 depletion moderately inhibited PE-induced cardiac myocyte hypertrophy (Figure S4C). Even more apparent was the fact that PE treatment strongly enhanced protein synthesis in control cardiomyocytes, whereas Dpr1-depleted cells completely blocked PE-induced protein synthesis, indicating a crucial role of Dpr1 in PE-mediated cardiomyocyte hypertrophy (Figure S4D).

Discussion

Here, we demonstrate that in cardiac myocytes Dpr1 activates rather than inhibiting canonical Wnt signaling, which is sufficient to induce hypertrophy in cardiac tissue and in isolated cardiomyocytes. In our Dpr1-tg mouse model, cardiac hypertrophy went along with stabilized β-catenin and robust expression of canonical Wnt target genes. These observations are in agreement with earlier reports of a relationship between β-catenin accumulation and cardiomyopathy.9–11 Rezvani and Liew12 observed post-translational regulation of β-catenin by adenomatous polyposis coli in hypertrophic hearts. Mice expressing a nondegradable form of β-catenin developed dilated cardiomyopathy and died after 5 months of age.13 After transverse aortic constriction, heart weight and cardiomyocyte width were significantly smaller in mice with cardiac-specific conditional β-catenin depletion.9

Contrary to our findings, Baurand et al14 observed that cardiac-restricted β-catenin depletion in mice was required for adaptive cardiac hypertrophy, whereas heart-specific stabilization of β-catenin reduced cardiac growth. However, in their study, depletion or stabilization of β-catenin is not a result of upstream Wnt signaling transduction but is rather an exclusive modification attributable to β-catenin expression. Transduction of the Wnt cascade, from ligand/receptor binding to target gene expression, recruits various cofactors and transcription factors that may play crucial roles in eliciting cardiac hypertrophy.

Dpr1 overexpression increased Dvl2 protein and mRNA abundance. Although the Dvl proteins exist in 3 isoforms (Dvl1–Dvl3) in mammalian cells, their quantitative distribution is not balanced. Dvl2 constitutes >95% of the total Dvl1-3 pool in mouse F9 cells and 80% of the pool in mouse P19 and human embryonic kidney 293 cells. However, modification of Dvl2 expression has the least effect on canonical Wnt signaling.7 Previous studies characterized a functional interaction between Dpr1 and Dvl2 in modulating Wnt activity. This occurs through hydrogen bonds between their PDZ domains.7 However, their combined role in transmitting Wnt signals is still unexplored. Overexpression of Dpr1 in HeLa cells blocks Dvl2-induced activation of the canonical Wnt cascade. In contrast, Dpr1

Figure 3. Dapper-1 (Dpr1) activates canonical Wnt signaling pathway in the murine heart. A, Western blot of left ventricular tissue using dishevelled (Dvl)1-, Dvl2-, and Dvl3-specific antibodies. Expression of Dvl2 protein and mRNA levels were increased in Dpr1-tg mice. B, Western blot showing increased protein levels of total-β-catenin, active-β-catenin, but decrease of β-catenin (pS33/37, pT41). C, Fluorescence image of tissue slide obtained from left ventricle showing translocation of β-catenin (red) into the nucleus in Dpr1-tg mice. ABC indicates active β-catenin. D, Increase of β-catenin target genes c-myc, peroxisome proliferator-activated receptor (PPAR)-δ, cyclinD1, and myoD. All data are presented as mean±SEM; *P<0.05 vs WT, n=7 animals each group for protein analysis; n≥10 animals each group for RNA analysis.
seems to be an essential Dvl cofactor for induction of the secondary axis in *Xenopus* embryos, a common feature to verify canonical Wnt signaling activity. In *Xenopus*, Dpr1 is required for notochord formation by antagonizing Wnt/β-catenin and JNK signaling. Downregulation of Dpr1 in human hepatocellular carcinoma cells correlates with accumulation of β-catenin, indicating that Dpr1 acts as a canonical/β-catenin Wnt inhibitor.

In Dpr1-tg mice, upregulation of Dvl2 was accompanied by stabilized β-catenin, its translocation into the nucleus, and increased β-catenin target gene expression. On the basis of this, we propose that canonical Wnt signals are transduced by Dvl2. In Dpr1-depleted cardiomyocytes, inhibition of the canonical Wnt pathway was associated with downregulation of Dvl2, suggesting Dpr1 as a crucial cofactor exclusive to Dvl2-mediated Wnt signaling (for suggested pathway, see Figure 6). Conversely, knockdown of Dvl2 did not alter Dpr1 abundance, indicating that Dpr1 operates upstream of Dvl2 in the Wnt cascade. This hypothesis is further supported by our observation that Dpr1 overexpression in Dvl2-depleted cardiomyocytes failed to activate TOPflash activity. Although Wnt3a-induced Wnt activity was blocked in Dvl2-depleted cells, endogenous Wnt signaling remained active, which is indicated by unaltered activated β-catenin levels (Figure 5B). This may be attributable to the redundancy of Dvl isoforms (Dvl1/Dvl3 may compensate for the absence of Dvl2, at least under baseline conditions). Our findings are supported by the results of a recent published study, where Dpr1 was suggested as a positive regulator of canonical Wnt signaling in colon cancer cells.

**Figure 4.** Dapper-1 (Dpr1) is necessary and sufficient to induce canonical Wnt signaling in cardiomyocytes. A, Representative Western blot analysis of Dpr1 knockdown in cultured cardiomyocytes. Expression of Wnt proteins dishevelled (Dvl)2, β-catenin, active β-catenin (ABC), and c-myc is reduced in Dpr1 knockdown cells. B, Representative Western blot demonstrating that lysosomal inhibition with NH4Cl (2.5 mmol/L) blocks Dvl2 degradation in Dpr1-depleted cells. C, Western blot demonstrating that knockdown of Dpr1 inhibits Wnt3a-induced activation of β-catenin in cardiomyocytes. D, Immunostaining with β-catenin specific antibody shows Wnt3a-induced translocation of β-catenin into the nucleus. In Dpr1 knockdown cells, translocation of β-catenin into the nucleus is blocked. E, Increased TOPflash reporter activity after stimulation with Wnt3a-conditioned medium. Knockdown of Dpr1 inhibits Wnt3a-induced activation of the Wnt signaling pathway. F, Adenoviral overexpression of Dpr1 synergizes the stabilizing effect of Wnt3a on β-catenin. G, Overexpression of Dpr1 increases TOPflash reporter activity under basal conditions. Stimulation with Wnt3a-conditioned medium shows synergistic effect. All data are presented as mean±SEM of ≥3 individual experiments in duplicate; *P<0.05 vs unstimulated control; #P<0.05 vs Wnt3a-stimulated control; Ad indicates adenovirus; L-cell, supernatant from L-cell culture nonexpressing Wnt3a was used as control; and pDC316-Dpr1, vector encoding Dpr1.
This study highlights that single Wnt components have a tissue- and context-dependent function. Although overexpression of Dpr1 in HeLa cells promotes lysosomal Dvl2 degradation, the situation in cardiac tissue and isolated cardiomyocytes is contrary. The crucial role of the Wnt pathway in development, cell differentiation, and cell proliferation may provide an explanation because coordination of these sensitive processes requires flexible properties in a subset of Wnt components. Thus, the complex role of Wnt members, especially in the process of cardiac remodeling, is still a challenging field to investigate in future studies.

Perspectives

This study demonstrates that cardiac-restricted overexpression of Dpr1 activates the canonical Wnt signaling pathway in a Dvl2-dependent manner. Our observations of Dpr1-depleted cardiomyocytes suggest that Dpr1 is a necessary and sufficient modulator of Wnt3a-mediated activation of the canonical Wnt branch. Moreover, stimulation of this pathway alone is sufficient to induce hypertrophy in cardiac tissue and in cultured cardiomyocytes. Blocking this pathway may thus serve as a novel therapeutic strategy for governing myocardial remodeling processes and alleviating cardiac hypertrophy.

Figure 5. Dapper-1 (Dpr1)-induced activation of canonical Wnt signaling is dishevelled (Dvl2)-dependent. A and B, Representative Western blot and bar graph showing Dvl2 overexpression in cardiomyocytes cultured in hypoxia conditions. C, Dpr1 was coimmunoprecipitated with Dvl2 in protein lysate of cultured cardiomyocytes. D, Representative immunoblot confirming Dvl2-specific siRNA knockdown reveals unaltered levels of Dpr1 and active β-catenin (ABC). E, ABC-specific immunoblot from Dvl2-depleted and Wnt3a-stimulated cardiomyocytes. F, Wnt3a-induced activation of β-catenin is inhibited in Dvl2-depleted cells. G, TOPflash reporter assay demonstrates that there is no interaction between β-catenin and T-cell factor transcription factors after knockdown of Dvl2. H, Dpr1-induced TOPflash activity is inhibited in Dvl2 knockdown cardiomyocytes. All data are presented as means±SEM of 3 individual experiments in duplicate; *P<0.05 vs unstimulated control; #P<0.05 vs Wnt3a-stimulated control. Ab indicates antibody; CoIP, coimmunoprecipitation; and L-cell, supernatant from L-cell culture nonexpressing Wnt3a was used as control.

Figure 6. Suggested role of Dapper-1 (Dpr1) in canonical Wnt signaling and cardiomyocyte hypertrophy. Dpr1 supports dishevelled (Dvl2)-mediated disruption of the β-catenin destruction complex. In turn, stabilized β-catenin translocates to the nucleus and interacts with transcription factors of the T-cell factor/lymphoid enhancer factor (TCF/LEF) family. This interaction induces expression of β-catenin target genes and cardiomyocyte hypertrophy. PPAR indicates peroxisome proliferator-activated receptor.
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Disclosures
None.

References

Novelty and Significance

What Is New?
- In search of a novel inhibitory strategy against temporal activation of Wnt signaling on myocardial stress, we examined the role of Dapper-1 (Dpr1) for activities of Wnt-signaling pathways. We report that Dpr1 does not antagonize but activates canonical Wnt signaling in cardiomyocytes. Activation of this pathway induces cardiac remodeling.

What Is Relevant?
- In our study, we first characterized the cardiac function and cardiac morphology of a Dpr1 transgenic mouse model. Here, we observed activation of canonical Wnt signaling accompanied by moderate cardiac hypertrophy. To gain further mechanistic insights, we studied the function of Dpr1 in isolated cardiomyocytes by siRNA-mediated knockdown or by adenoviral overexpression. The in vitro results were congruent with the in vivo findings and, moreover, we provide evidence that knockdown of Dpr1 inhibits phenylephrine and Wnt3a-induced hypertrophy in cardiomyocytes.

Summary
Our study demonstrates for the first time that Dpr1-mediated activation of canonical Wnt signaling is necessary and sufficient to induce cardiomyocyte hypertrophy. Inhibition of this pathway may thus serve as a novel therapeutic strategy for governing myocardial remodeling processes and alleviating cardiac hypertrophy.
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Dapper-1 induces myocardial remodeling through activation of canonical Wnt signaling in cardiomyocytes

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Supplemental Materials and Methods

**Myocardial infarction**
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). For the MI model we used male Wistar rats with a weight of 200 g (Charles River Laboratories, Sulzfeld, Germany). Induction of MI was performed as described previously. Expression of Dpr1 was assessed 1 week after MI induction.

**Dpr1 transgenic mouse (Dpr1-tg) model**
Murine Dpr1 cDNA was cloned into an α-myosin-heavy-chain promoter construct. This construct was microinjected into C57BL/6 mouse embryos, and PCR and Southern blotting were used to screen for founders and Dpr1-tg mice. Eight transgenic founders were born alive. The founder with the most over-expression was used as the working line.

**Echocardiography**
Mice were anesthetized by isofluorane inhalation (~2% isofluorane) for echocardiography. M-mode measurements of the LV dimensions were taken, with at least three readings per mouse. LV percent fractional shortening was calculated as LVFS = [(EDD-ESD)/EDD]³ x 100, where EDD is the end-diastolic diameter and ESD is the end-systolic diameter. The observer was blinded to the genotype of the mice.

**Pressure-volume loop analysis**
This method is based on measuring the time-varying electrical conductance signal of two segments of blood in the LV, allowing calculation of the total LV volume. Raw conductance volumes were corrected for parallel conductance by the hypertonic saline dilution method. Data were digitized with a sampling rate of 1000 Hz and recorded on a personal computer using the Chart software package (AD Instruments). Subsequent analysis of pressure-volume loops was carried out with the PVAN software (Millar Instruments, Inc.).

**Histopathology**
Mice were euthanized; the LV’s were isolated, fixed in formalin overnight, paraffin embedded, and processed for sectioning. We made 0.5µM sections from the LV samples and stained them with H&E to determine the myocyte size.

**siRNA Transfection**
For at least 5 h myocytes were cultured in serum and antibiotic free medium. For transfection of 2 × 10⁵ cardiomyocytes in a 6 cm dish 250 pmol siRNA was diluted in 650 µL serum- and antibiotic free OptiMEM (Invitrogen). In another tube 4.5 µL of Lipofectamin 2000 (Invitrogen) was diluted in 200 µL OptiMEM and incubated for 15 min. After complex formation the solution and OptiMEM were added to the cells. The cells were incubated for 72 h at 37 °C. siRNAs used were Dpr1 5'-GCAGAUAAGUGACCUGAGA-3', Dvl2 5'-GUACCGAGAUUC-3', and non-specific control siRNA 5'-AGGUAGUGUAUCGCCUUGTT-3'. In all siRNA mediated knockdown experiments myocytes transfected with non-specific (scrambled) siRNA were used as controls. siRNAs were synthesized by MWG (Ebersberg, Germany).

**Cardiomyocyte culture and determination of myocyte surface area**
Isolation and culture of cardiac myocytes was performed as described previously. For determination of surface area, cardiomyocytes were cultured on coverslips and transfected with siRNA. Forty-eight hours later, the cells were stimulated with 50µM phenylephrine (PE) or Wnt3a for further two days. Myocytes were washed twice with ice-cold PBS and fixed in PBS containing 4% paraformaldehyde for 30 min. For permeabilization, cells were incubated for 10 min in PBS containing 0.1% Triton X. Fixed cells were blocked with 2% BSA in PBS.
for one hour. Immunostaining was performed with Texas Red phalloidin (Molecular Probes) and Hoechst 33342 for 30 min. The coverslips were fixed onto microscope slides after staining. For the calculation of myocyte surface area, microscope images were captured with a digital camera. Analysis was performed using Image J.

**RNA analysis**
RNA was isolated with Trizol® reagent according to the manufacturer's protocol. Real-time PCR was performed using the Universal Probe Library from Roche Diagnostics. Primers and probes used are HPRT 5’-gtcaaggggcataaaag-3’ and 5’-tgcatgttttacaggcttaa-3’, probe #22; Dpr1 5’-gatgtggagaagacatctgaagag-3’ and 5’-tccatacgcagctataaaacc-3’, probe #80; ANF 5’-caacacagatctgatggtgattca-3’ and 5’-cccatctctacgggcatc-3’, probe #25; BNP 5’-cttcctctccccacacgc-3’ and 5’-aagagacccacaggcaatc-3’, probe #109; β-MHC 5’-caaggtcaatactctgaccaagg-3’ and 5’-ccatgcgcactttctctc-3’, probe #78; Dvl2 5’-acttcaccacttctggaa-3’ and 5’-gaggagcaggtaagcag-3’, probe #42; β-catenin 5’-gacgaggttggtgag-3’ and 5’-tggagagctccagacc-3’, probe #5; cdc1 5’-gagattgtgcctcatgc-3’ and 5’-ctctctcttgacttcttgc-3’, probe #67; c-myc 5’-cctctctcttgacttcttgc-3’ and 5’-ccatctctatctcttgc-3’, probe #77; PPARδ 5’-gatgacgctgggactac-3’ and 5’-gtgtggagcagatggag-3’, probe #15; myoD 5’-aagcactagttgcgactca-3’ and 5’-ggccgctgtaatccatcat-3’, probe #42.

**Protein analysis**
Immunoblotting was performed as described previously ². The primary antibodies used in this investigation were anti-Dapper1 (mouse; Abcam), anti-Dapper1 (rat; Santa Cruz Biotechnology), anti-Dvl1 (Santa Cruz Biotechnology), anti-Dvl2 (Cell Signaling Technology), anti-β-catenin (Santa Cruz Biotechnology), anti-active-β-catenin (Millipore), anti-phospho-β-catenin (Cell Signaling Technology), anti-c-myc (Cell Signaling Technology), anti-α-actin (Sigma-Aldrich), and anti-GAPDH (United States Biological). HRP-conjugated anti-rabbit IgG, anti-mouse IgG, and anti-goat IgG antibodies (Santa Cruz Biotechnology) were used as secondary antibodies. Bands were quantified by densitometry using Image J.

**Co-immunoprecipitation**
Whole cell extract from isolated neonatal cardiomyocytes were prepared in IP lysis buffer (Pierce, Rockford, USA). Lysate was precleaned with protein A agarose beads for 2h at room temperature and then incubated with antibodies against Dvl2 and protein A agarose beads at 4°C over night. After three washing steps with lysis buffer the immunocomplex was boiled for 5min at 99°C. After cooling down, solution was loaded on a SDS-PAGE.

**Immunofluorescence**
For immunofluorescence analysis, cardiomyocytes were fixed with 3.7% formaldehyde, followed by incubation steps with the primary and secondary antibody (Anti-β-catenin antibody and Anti-mouse Cy3 labeled antibody, Santa Cruz; Anti-Dpr1 antibody and Anti-Rabbit Texas-Red labeled antibody, Abcam). For counterstaining DNA we used Hoechst 33342 probes (Invitrogen, Eugene, Oregon, USA). The fluorescence signals were detected and visualized by using a Nikon A1Rsi 32 channel spectral imaging confocal laser scanning system on a Nikon Ti inverted microscope.

**TOPflash assay**
Cardiomyocytes were cultured in serum free medium in 6-well plates. The next day, siRNA transfection was performed as described previously ². Twenty-four hours later, cardiomyocytes were co-transfected with TOPFlash reporter and renilla luciferase pRL-TK Vectors according to the manufacturer’s protocol (Millipore) for 48 hours. 24hours before harvesting cells were incubated with Wnt3a conditioned medium. TOPFlash activity was measured using a dual luciferase reporter system (Promega) and normalized to renilla luciferase activity.
**Adenoviral infection of isolated cardiomyocytes**
Cardiomyocytes were cultured with serum free medium in 6 well plates. The next day cardiomyocytes were infected over night with adenovirus encoded Dpr1 (Applied Biological Materials Inc., Richmond, Canada) or an empty adenovirus vector as a control. After washing the cells 3 times with PBS the culture medium was changed. 48h later the cardiomyocytes were stimulated with Wnt3a conditioned medium 4h before isolation of the proteins and determination of active β-catenin levels.

**[3H]-glycine incorporation assay**
Cardiomyocytes were cultured in 12-well plates. The next day, cells were transfected with the appropriate siRNA for 48 hours. Then, cells were stimulated with Wnt3a conditioned medium or PE and incubated with 1 µCi/ml [3H]-glycine overnight. Cells were washed twice with ice-cold PBS, and proteins were precipitated for one hour with 10% trichloroacetic acid in a 4°C cold freezer. The precipitate was dissolved in 1 ml 1M NaOH. [3H]-glycine incorporation was determined by scintillation counting.

**Statistical analysis**
Data are reported as the mean ± standard error of the mean (SEM). Between-group differences were assayed by a two-tailed Student’s t-test or by multiple ANOVA, as applicable. A post hoc test was performed by the method of Tukey. Significance was accepted at the P<0.05 level.

**Supplemental References**


Figure S1: Robust over-expression of Dpr1 in a rat myocardial infarction model. (A/B) Bar graphs showing increased Dpr1 protein and mRNA levels (n=5 animals in each group). (C/D) Bar graphs showing increased Dpr1 protein and mRNA in cardiomyocytes cultured in hypoxia conditions. (H) Western Blot confirming over-expression of Dpr1 in Dpr1-tg mouse model. (All data are presented as mean ± SEM; *p<0.05 vs. sham animals or normoxia cells, respectively; n=3 individual experiments in duplicates in hypoxia experiments)
Expression of Dvl2 and β-catenin in Dpr1-tg mice

(A/B) Expression of Dvl2 protein and mRNA levels were increased in Dpr1-tg mice. (C) Increased protein levels of total-β-catenin, active-β-catenin but decrease of β-catenin [pS33/37, pT41] was observed. (D) Unaltered mRNA abundance of β-catenin indicates posttranslational stabilization (All data are presented as mean ± SEM; *p<0.05 vs. wt, n=7 animals each group).
Dpr1 is necessary and sufficient to induce canonical Wnt signaling in cardiomyocytes

(A) Bar graph showing protein levels of Dvl1, Dvl2 and Dvl3 after Dpr1 depletion. (B) Unaltered mRNA levels of Dvl2 following Dpr1 knockdown. (C) Lysosomal inhibition with NH4Cl blocks Dvl2 degradation in Dpr1 depleted cells. (D) Levels of β-catenin, ABC and c-myc are reduced in Dpr1 knockdown cells while (E) mRNA transcription β-catenin is unaltered. (F) Immunostaining with Dpr1 specific antibodies revealed nuclear translocation following Wnt3a stimulation. (G) Bar graph shows stabilisation of ABC following Wnt3a stimulation. Knockdown of Dpr1 inhibits this effect. (H) Adenoviral over-expression of Dpr1 increases the amount of activated β-catenin under basal conditions and synergizes the stabilizing effect of Wnt3a stimulation. (All data are presented as mean ± SEM of at least three individual experiments in duplicates; *p<0.05 vs. unstimulated control; #p<0.05 vs. Wnt3a stimulated control; ABC means active β-catenin)
Knockdown of Dpr1 inhibits Wnt3a- and PE-induced cardiomyocyte hypertrophy. (A/B) Increase in myocyte surface area and protein synthesis following stimulation with Wnt3a-conditioned medium. Knockdown of Dpr1 inhibits Wnt3a-induced cardiomyocyte hypertrophy. Data are presented as mean ± SEM of three individual experiments; *P<0.05 vs. unstimulated control, #P<0.05 vs. Wnt3a-stimulated control. (C) PE-induced enlargement of cardiomyocyte surface is attenuated in Dpr1-depleted cells. (D) PE-mediated activation of protein synthesis is blocked in Dpr1 knockdown cardiomyocytes. (All data are presented as mean ± SEM of three individual experiments; *P<0.05 vs. unstimulated control; #P<0.05 vs. PE-stimulated control; PE, phenylephrine; L-cell, supernatant from L-cell culture non-expressing Wnt3a was used as control)