Supporting Information

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SI Materials and Methods

Microarray and qPCR. Total RNAs were extracted from hearts or cells with TRIzol reagent (Invitrogen) or RNeasy Kit (Qiagen) according to the manufacturer's instructions, respectively. cDNA was synthesized using RT2 HT first-strand kit (Qiagen) with 2 μg of RNA as a template. qPCR was performed using Lightcycler 480 (Roche). Relative fold-change was calculated using the ΔΔCt method after normalizing to Gapdh. Microarray analysis was performed by the University of Texas Southwestern Microarray Core facility using the MouseWG-6 V2.0 BeadChips (Illumina) using RNA extracted from heart samples and subsequently pooled before analysis.

MI and Drug Treatment. C57BL/6, 12-wk-old male mice, underwent permanent ligation of the LAD. Adult mice were anesthetized with isoflurane. Thoracotomy was performed at the third intercostal space, and self-retaining microretractors were placed to separate the third and fourth rib to visualize the LAD. The LAD was surgically ligated without tearing the pericardial sac. After LAD ligation, the retractors were removed and the chest was closed. Wnt-974 was administered by oral gavage at 5 mg/kg per mouse once per day for 10 wk.

Cardiac MRI. The cardiac function of mice was evaluated by cardiac MRI using a 7T small-animal MR scanner [Agilent (Varian)]. Under anesthesia by inhalation of 1.5–3% (vol/vol) isoflurane, the animals were placed prone on a mouse sled (Dazai Research Instruments) equipped with a pneumatic respiratory sensor and ECG electrodes for cardiac sensing, head first, with the heart centered with respect to the center of the RF coil. The chest area was shaved and a conducting gel was applied to optimize ECG contact between electrodes and mouse. All MRI acquisitions were gated using both cardiac and respiratory triggering. The bore temperature was kept at 33 ± 2 °C to assure adequate and constant heart rate. Axial images perpendicular to the long axis of the heart were chosen for Cine-imaging. Each scan consisted of five to nine contiguous slices from apex to left ventricle (LV) outflow with 1-mm thickness. Epicardial and endocardial borders were manually traced for calculation of left ventricular end systolic and end diastolic volumes (LVESV and LVEDV) using NIH ImageJ (v1.47j) software. Total LV volumes were calculated as the sum of all slice volumes. The LV ejection fraction (LVEF) was calculated by the equation, (LVEDV − LVESV)/LVEDV × 100%. Investigators performing MRI analysis were blinded to the assignment of mice in each group.

Histology and Immunostaining. Tissues were fixed in 4% (wt/vol) paraformaldehyde, embedded in paraffin, and cut 5-μm thick. For cryosection, hearts were fixed in 4% (wt/vol) paraformaldehyde for 1 h at room temperature and incubated in 30% (wt/vol) sucrose/PBS. Tissues were embedded in OCT medium, frozen at −80 °C. Cardiac fibrosis was evaluated by trichrome and picrosirius red staining 11-wk after MI injury. Heart sections were obtained starting at the level of 0.5 mm above the suture area and collected at three additional levels at a 1-mm interval. The stained sections were scanned by Artix Scan 4000tf. The images of the middle three levels were processed in Adobe Photoshop CS6 and analyzed in ImageJ to quantify blue-stained fibrotic areas. For immunostaining, sections were subjected to antigen retrieval and followed by staining against antibodies PCNA (Sigma; 1:100), cTnT (Millipore; 1:100), PH3 (Santa Cruz Biotechnology; 1:100), Col6a3 (Fitzgerald; 1:50). Conjugated antibodies (Alexa Fluor488 and 594, Invitrogen) were used as a secondary antibody. Images were taken with a Leica Microsystem 5500 microscope.

Cell Culture, Recombinant Wnt Proteins, and Lentiviral Transduction. Human CMs differentiated from human stem cells were provided by S.P.P. (University of Wisconsin at Madison, Madison, WI) and were grown in DMEM supplemented with 20% (vol/vol) FBS, in a 37 °C humidified incubator containing 5% CO_2. Human cardiac microvascular endothelial cells (Lonza) and human cardiac fibroblasts-adult ventricular (ScienCell) were cultured according to the manufacturers' instructions. After 16 h of serum starvation, cells were incubated for 4 h in the presence of 100 ng/mL recombinant mouse Wnt3a (Time Bioscience). Human WNT3-Lentiviral vector (pLV-mCherry:T2A:Puro-EF1A > hWNT3) was produced by VectorBuilder. Virus-containing media were collected after transfection into HEK-293T cells and used for transduction in the presence of >1 μg/mL Polybrene (Sigma).

Western Blot Analysis. Total cell lysates were prepared by lysing the cells with 2× sample loading buffer containing protease inhibitor mixture (Roche) and 2.5% (vol/vol) beta mercaptoethanol (Sigma). SDS/PAGE was conducted with antibodies against Wnt5a/b and Dvl2 (Cell Signaling Technology; 1:1,000), Wnt3 and Col6a3 (Santa Cruz Biotechnology; 1:1,000), β-actin (Sigma; 1:2,000). A LiCor Odyssey Fc instrument was used to detect chemiluminescent signal.

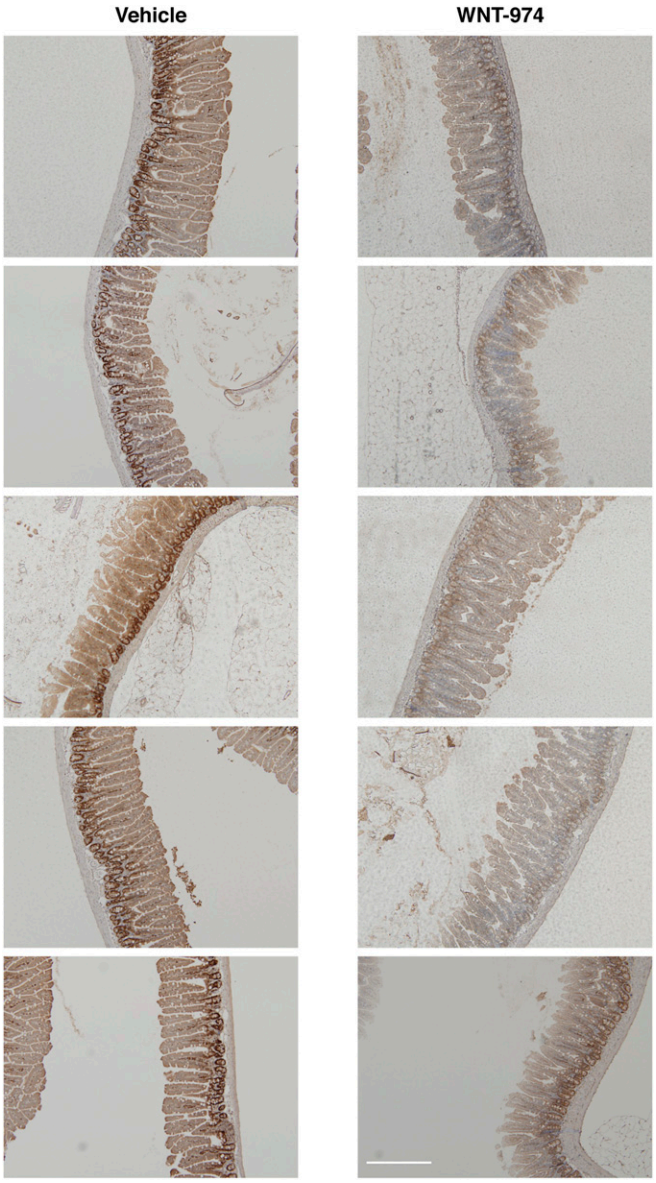

Fig. S1. Images of PCNA staining in gut tissue isolated from WNT-974 or vehicle-treated mice. (Scale bar, 200 μm.)

A	TV	BV	BV/TV	Mean/Density [mg HA/ccm] of BV (Material)	cortical thickness	cortical porosity
WNT974	0.3544	0.3431	0.9683	1006.1505	0.131	0.0317
WNT974	0.3725	0.3621	0.9722	1119.0068	0.148	0.0278
VEHICLE	0.4858	0.4771	0.982	1095.4897	0.207	0.018
VEHICLE	0.5385	0.5285	0.9813	1144.3771	0.204	0.0187
WNT974	0.3853	0.3753	0.974	1061.0449	0.147	0.026
VEHICLE	0.5079	0.499	0.9823	1141.5653	0.205	0.0177
WNT974	0.3685	0.3574	0.9701	1033.2463	0.155	0.0299
VEHICLE	0.509	0.4991	0.9806	1120.6045	0.198	0.0194
VEHICLE	0.4497	0.4415	0.9819	1080.4082	0.209	0.0181
WNT974	0.4167	0.4069	0.9764	1127.0588	0.154	0.0236

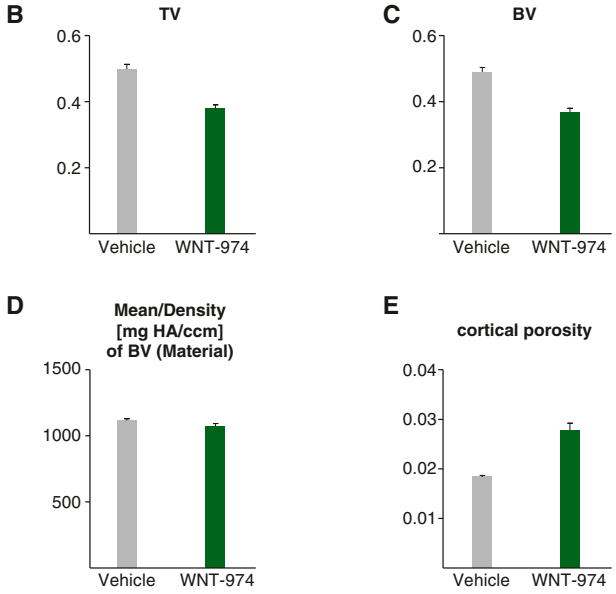

Fig. S2. Bone density measurements of tibia midshaft. (*A*) Data averaged from 200 sections of midshaft bone from mice treated with WNT-974 or vehicle generated using microcomputed tomography analysis. (*B*) Total volume (TV) of bone region measured. (*C*) Bone volume (BV) of region measured. (*D*) Density of BV. (*E*) Cortical porosity results.

A

Tibia metaphasis

	TV	BV	BV/TV	Mean/Density [mg HA/ccm] of BV (Material)
WNT974	1.1294	0.0325	0.0288	924.9912
WNT974	1.1369	0.0274	0.0241	921.093
VEHICLE	1.0802	0.0836	0.0774	922.1793
VEHICLE	1.1352	0.0945	0.0832	946.0798
WNT974	1.2217	0.0318	0.026	901.7936
VEHICLE	1.2302	0.1128	0.0917	920.6456
WNT974	1.0895	0.0718	0.0659	915.2776
VEHICLE	1.0953	0.0287	0.0262	937.8361
VEHICLE	1.3175	0.1187	0.0901	940.9674
WNT974	1.3011	0.0424	0.0326	938.4113

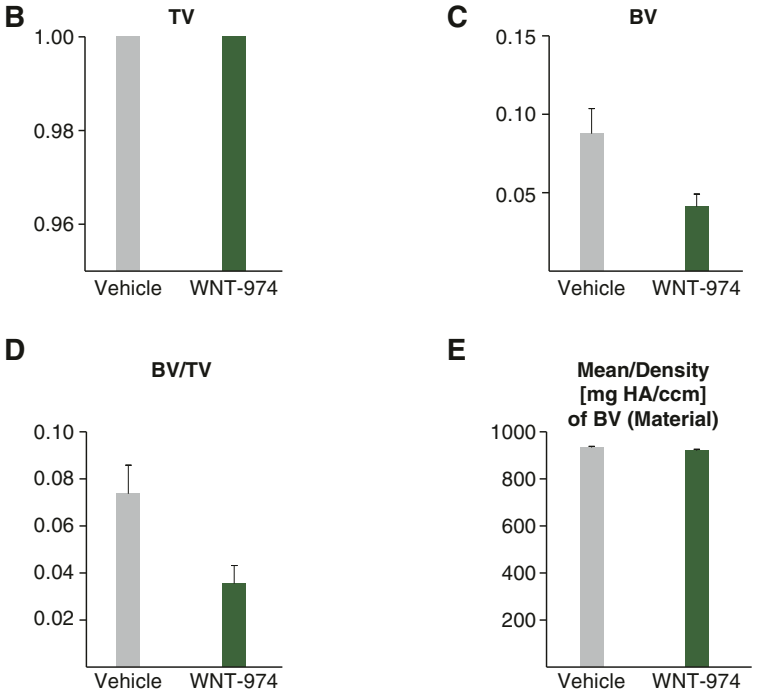

Fig. S3. Bone density measurements of tibia metaphysis. (*A*) Data averaged from 100 sections of metaphyses from mice treated with WNT-974 or vehicle generated using microcomputed tomography analysis. (*B*) Total volume (TV) of bone region measured. (*C*) Bone volume (BV) of region measured. (*D*) Ratio of BV/TV. (*E*) Density of BV.

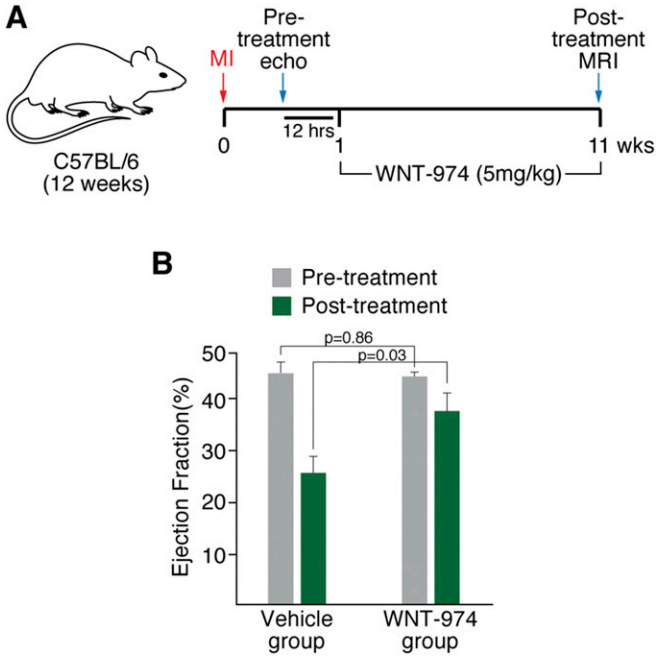

Fig. S4. Inhibition of Porcn improves heart function after LAD ligation. (*A*) Animals following LAD ligation with comparable heart function as determined using echocardiography ($n = 10$ per group) were dosed with either WNT-974 (5 mg/kg; 1× by mouth per day) or vehicle for 10 wk. Heart function of animals was then determined using MRI. (*B*) WNT-974–treated animals exhibit improved ejection fraction following LAD ligation.

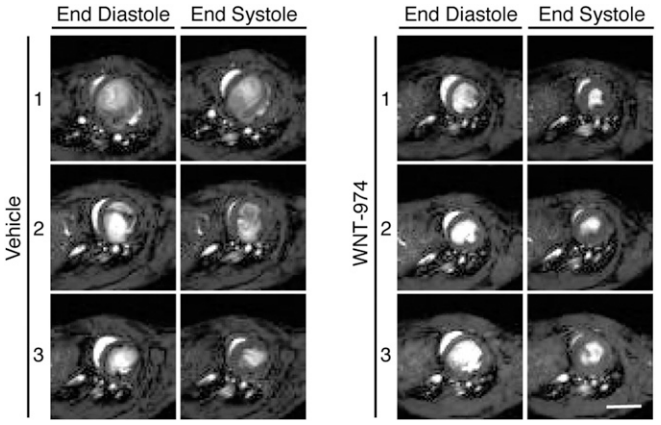

Fig. S5. MRI images of three mice treated with WNT-974 or vehicle. (Scale bar, 5 mm.)

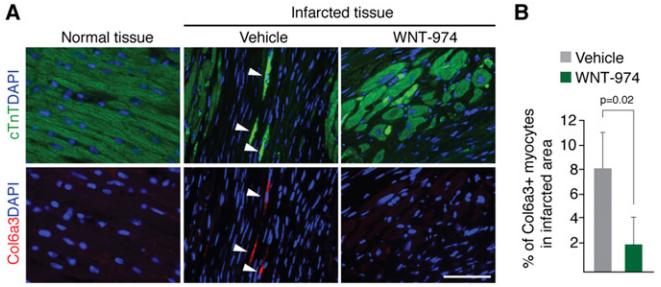

Fig. S6. Decreased expression of Col6a3 in cardiomyocytes exposed to WNT-974. (*A*) Heart tissue from LAD ligated animals were costained for Col6a3 and cTnT to identify the number of CMs expressing Col6a3 in the presence or absence of WNT-974. Normal tissue was derived from a remote area away from the site of infarction. (Scale bar, 50 μm.) (*B*) Quantification of Col6a3 positive myocytes in infarcted area of mice treated with WNT-974 or vehicle.

Table S1. Gene expression data for ER stress-related genes in heart tissue exposed to WNT-974 or vehicle derived from microarray analysis

Symbol	PROBE_ID	Vehicle	WNT-974	Fold-change	Acession	Source
Amfr	ILMN_2722902	1737.9	2027.6	1.17	NM_011787	Liu et al. (50)
Fbxo6b	ILMN_2660175	1881.4	1847.6	0.98	NM_015797.1	Yoshida et al. (51)
Herpud1	ILMN_2790246	3050.9	3209.5	1.05	NM_022331.1	Ma and Hendershot (52)
Mbtps1	ILMN_2748537	158.7	182.5	1.15	NM_019709.3	Patra et al. (53)
Nucb1	ILMN_2908735	359.9	343.7	0.95	NM_008749.1	Lavoie et al. (54)
Sel1l	ILMN_3137920	185.9	137.7	−1.35	NM_001039089.1	Sun et al. (55)
Syvn1	ILMN_2628258	1219.4	1294.1	1.06	NM_028769.4	Doroudgar et al. (56)

Table S2. MRI data for pre- and posttreated mice with WNT-974 or vehicle following LAD ligation

Group	Animal no.	EF (%)	HR (beat/min)	SV (μL)	CO(μL)	LVEDV(μL)	LVESV(μL)
Pretreatment (12 h before initial dosing)							
Vehicle	M2535	34.7	573.7	41.7	23.9	120.1	78.4
	M2541	19.2	563.2	31.4	17.7	163.9	132.4
	M2543	27.7	523.4	32.4	17.0	117.2	84.8
	M2553	28.7	523.9	36.5	19.1	127.1	90.5
	M2555	34.5	498.1	34.4	17.1	99.6	65.2
	M2557	43.5	519.7	44.1	22.9	101.6	57.4
	M2563	30.3	543.2	35.5	19.3	117.3	81.7
	M2564	21.9	551.8	35.8	19.8	163.4	127.5
	M2567	40.3	565.9	44.5	25.2	110.5	66.0
	Average	31.2	540.3	37.4	20.2	124.5	87.1
WNT974	M2530	49.3	458.0	36.4	16.7	73.8	37.4
	M2531	32.8	540.5	34.8	18.8	106.1	71.3
	M2534	47.1	550.4	42.4	23.3	89.9	47.6
	M2537	28.6	550.4	29.8	16.4	104.0	74.2
	M2542	38.4	588.1	28.6	16.8	74.6	46.0
	M2549	30.1	504.1	37.3	18.8	123.7	86.4
	M2550	29.7	545.3	35.3	19.2	118.8	83.6
	M2554	37.0	527.6	27.9	14.7	75.6	47.7
	M2556	37.2	526.3	27.5	14.5	74.0	46.5
	M2558	26.4	530.9	44.5	23.6	168.7	124.1
	M2560	31.2	559.2	40.2	22.5	128.9	88.7
	M2561	37.6	544.6	37.9	20.6	100.7	62.8
	M2562	32.5	521.6	36.1	18.8	111.1	75.0
	M2565	19.3	540.4	35.1	18.9	181.3	146.2
	M2568	32.3	534.9	35.3	18.9	109.0	73.7
	Average	34.0	534.8	35.3	18.8	109.3	74.1
Posttreatment (10-wk dosing)							
Vehicle	M2535	16.6	540.5	66.9	36.2	402.7	335.8
	M2541	21.8	576.7	49.8	28.7	228.5	178.7
	M2543	20.3	556.8	29.7	16.5	146.3	116.6
	M2553	30.2	582.4	54.4	31.7	179.9	125.5
	M2555	35.0	508.4	46.1	23.4	131.8	85.7
	M2557	20.9	582.4	36.1	21.0	173.1	137.0
	M2563	26.6	540.5	42.6	23.0	160.3	117.7
	M2564	23.6	479.8	48.9	23.5	207.5	158.6
	M2567	33.8	594.7	45.1	26.8	133.5	88.4
	Average	25.4	551.4	46.6	25.7	196.0	149.3
WNT974	M2530	61.4	483.8	48.7	23.6	79.4	30.7
	M2531	34.4	468.2	56.2	26.3	163.3	107.0
	M2534	52.5	559.9	57.5	32.2	109.5	52.0
	M2537	24.7	571.4	52.2	29.9	211.2	159.0
	M2542	56.2	479.6	47.9	23.0	85.2	37.3
	M2549	30.5	571.4	49.6	28.3	162.5	112.9
	M2550	20.6	576.7	28.0	16.2	136.1	108.1
	M2554	45.1	593.8	39.7	23.6	88.1	48.3
	M2556	26.6	550.4	25.1	13.8	94.3	69.2
	M2558	22.9	501.3	64.2	32.2	279.6	215.4
	M2560	42.6	333.1	77.8	25.9	182.6	104.8
	M2561	41.9	526.3	47.7	25.1	113.8	66.1
	M2562	41.0	523.5	50.0	26.2	122.1	72.1
	M2565	26.2	521.6	54.6	28.5	208.7	154.1
	M2568	36.1	576.7	45.3	26.1	125.4	80.1
	Average	37.5	522.5	49.6	25.4	144.1	94.5

Abbreviations: CO, cardiac output; EF, ejection fraction; HR, heart rate; LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume; SV, stroke volume.

Other Supporting Information Files

Dataset S1 (XLSX)