Supplemental Material for:

Differential Wnt-mediated Programming and Arrhythmogenesis in Right versus Left Ventricles

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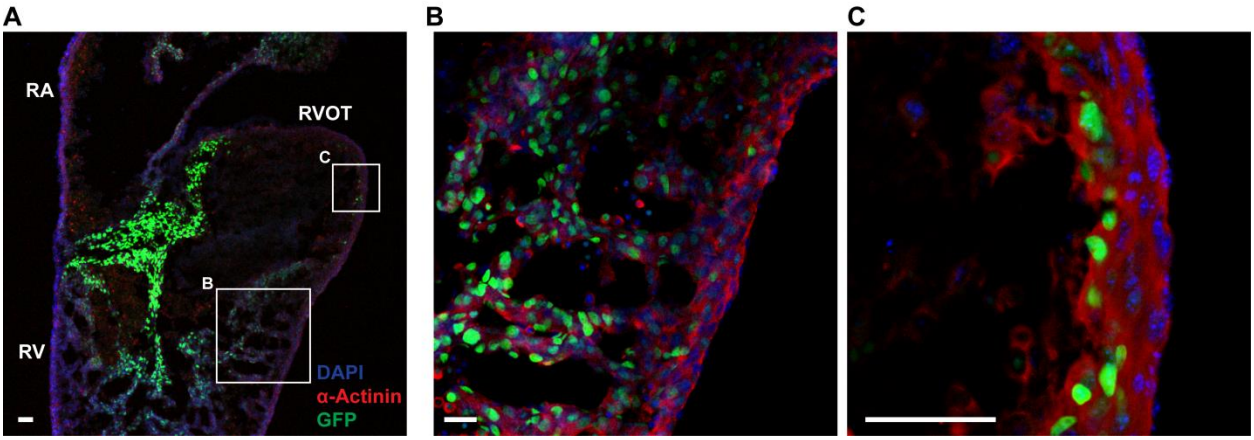
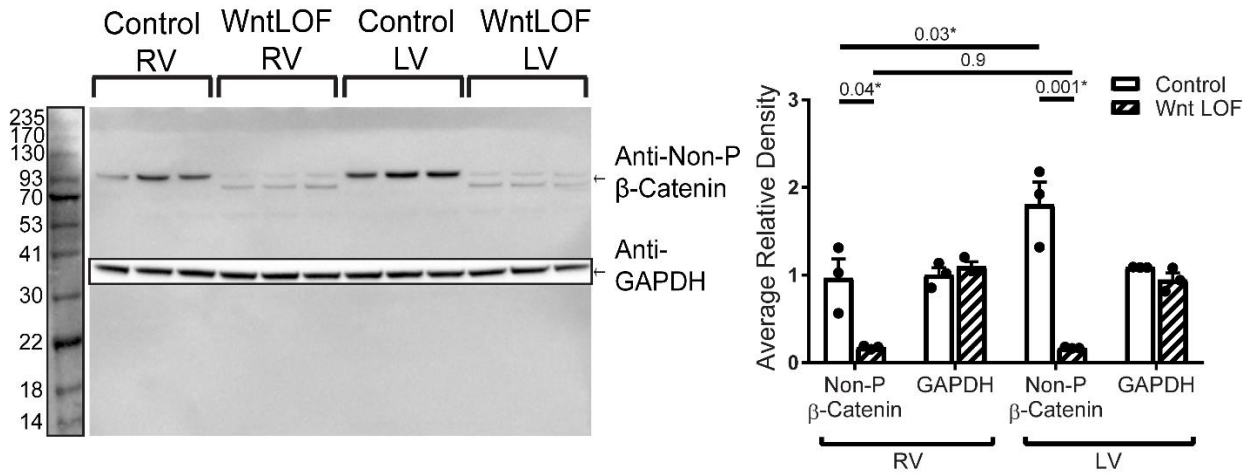
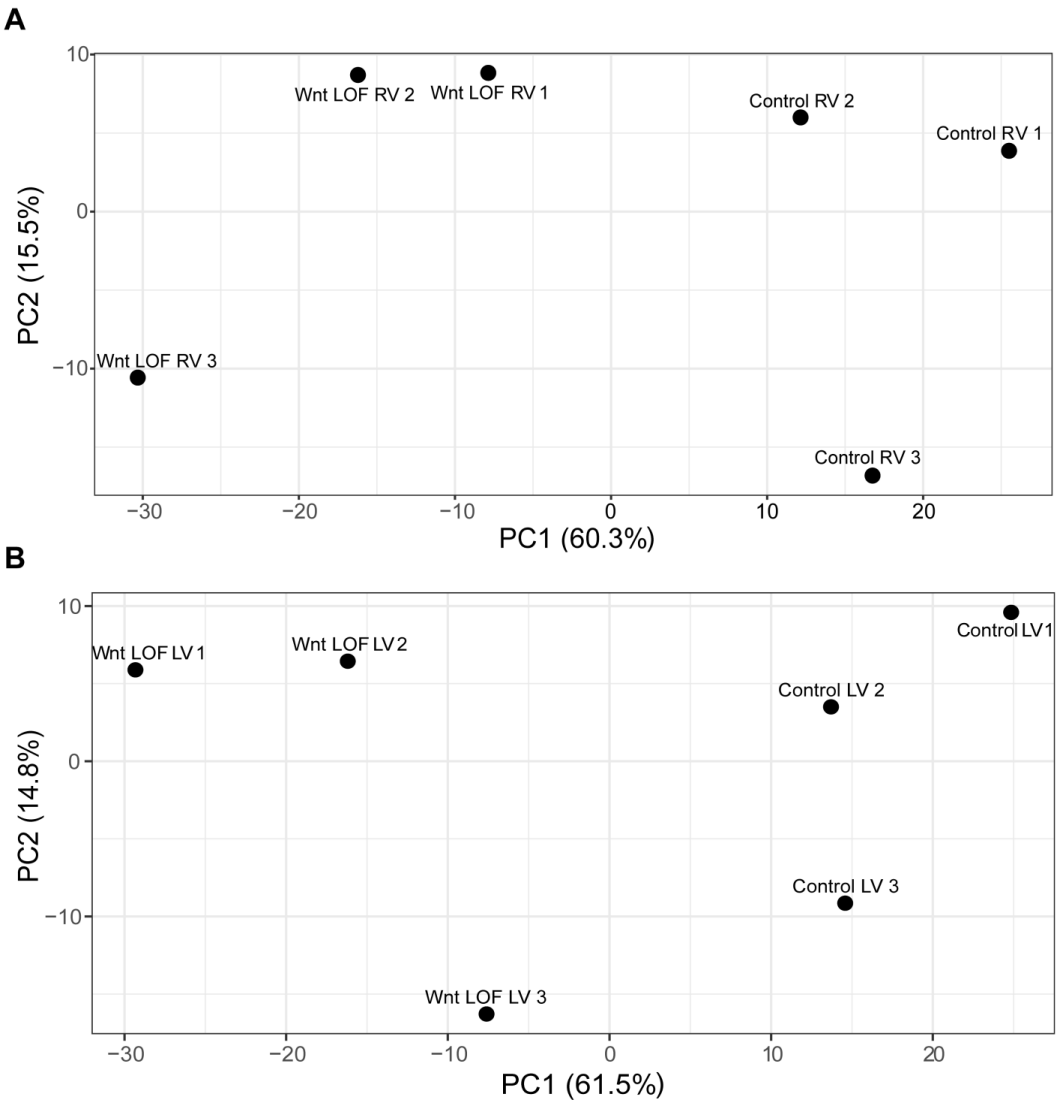
Supplemental Figures with Legends



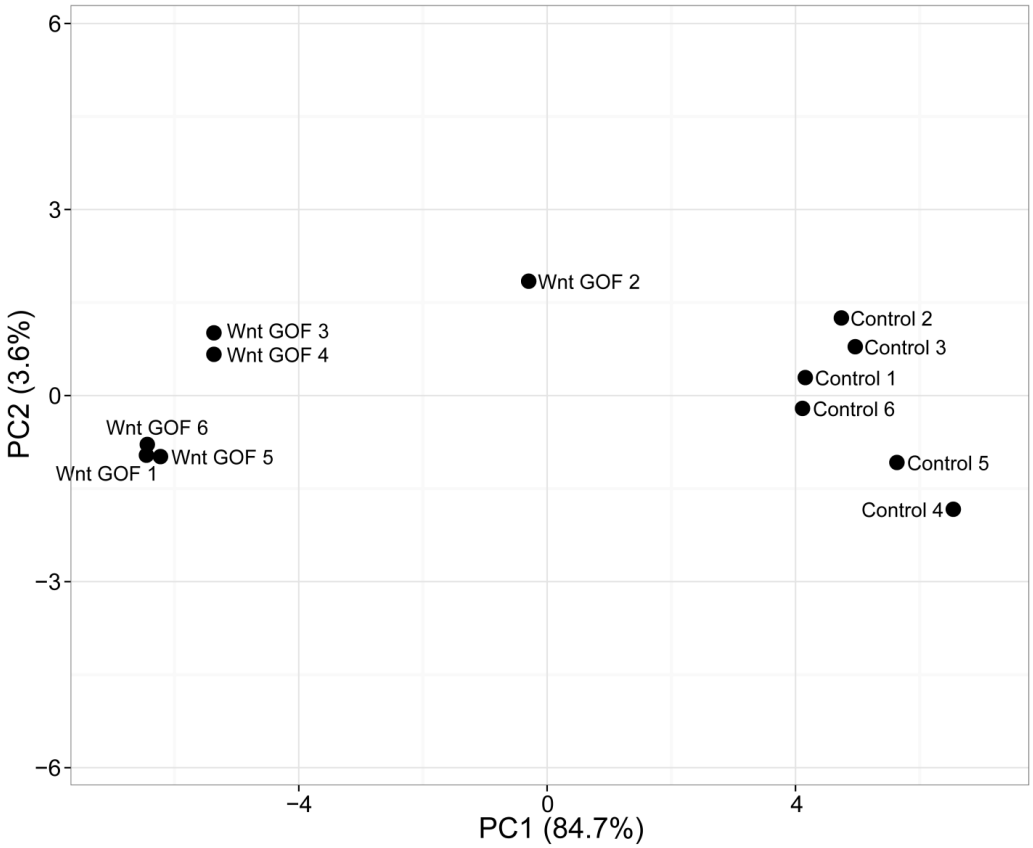
Supplemental Figure 1. Wnt signaling is active in RV and RVOT cardiomyocytes during development. (**A**) Sections from E12.5 TCF/Lef:H2B-GFP reporter mice show Wnt activity, as reported by nuclear GFP expression, is highest within the atrioventricular region. In addition, Wnt signaling is found in the right ventricle (RV) and right ventricular outflow tract (RVOT) in myocytes and non-myocytes. Immunostaining with α-actinin (red) and DAPI (blue) reveals a high percentage of cardiomyocytes have active Wnt signaling at this developmental stage. (**B**) Higher magnification view of the region within the RV from panel A shows higher GFP expression in cardiomyocytes of the trabecular region compared with the compact zone of the ventricular myocardium. (**C**) Higher magnification view of the RVOT region from panel A shows Wnt activity is active within RVOT cardiomyocytes. Scale bar in each image = 50 μm. RA=right atrium.



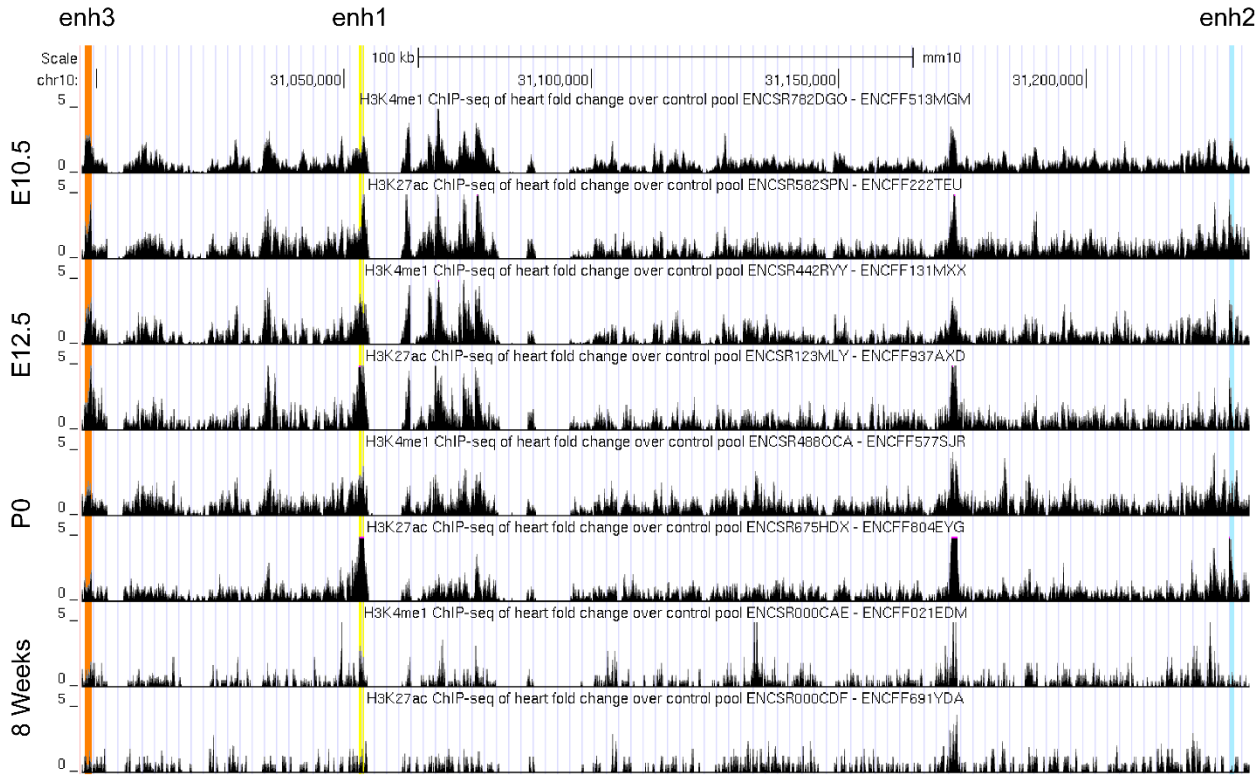
Supplemental Figure 2. Non-phosphorylated (active) β-catenin levels are higher in control left ventricle when compared with right ventricle. Western blot for non-phosphorylated (active) β-catenin in adult control (*β-cat*$^{fl/+}$) and littermate Wnt LOF (*Mlc2v*$^{Cre/+}$*; β-cat*$^{fl/\,DM}$) mice (n=3 each genotype). Wnt LOF mice express a truncated β-catenin protein at approximately 70kDa, in addition to wild type β-catenin at 93kDa, as expected from the loss of function model. GAPDH is used for normalization, and quantification of protein levels based on band density shows significantly reduced β-catenin in control RV compared to control LV. One-way ANOVA and post-hoc Tukey's test were performed to determine statistical significance. *P<0.05 was considered statistically significant.



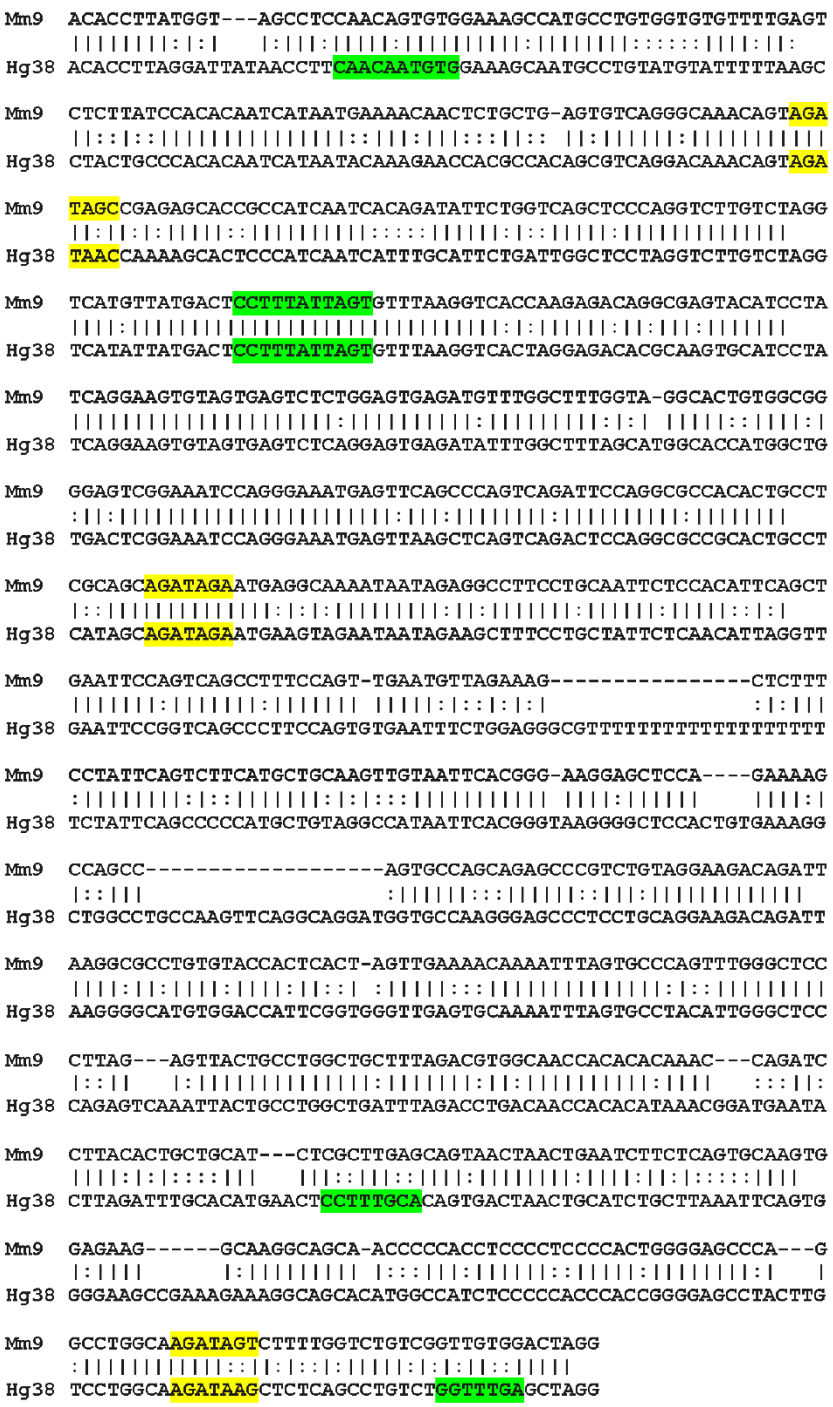
Supplemental Figure 3. Principal Component analysis of Wnt loss-of-function RNA-sequencing transcript results. Both Wnt LOF RV (**A**) and Wnt LOF LV (**B**) are separated from their respective controls in the first principal component.



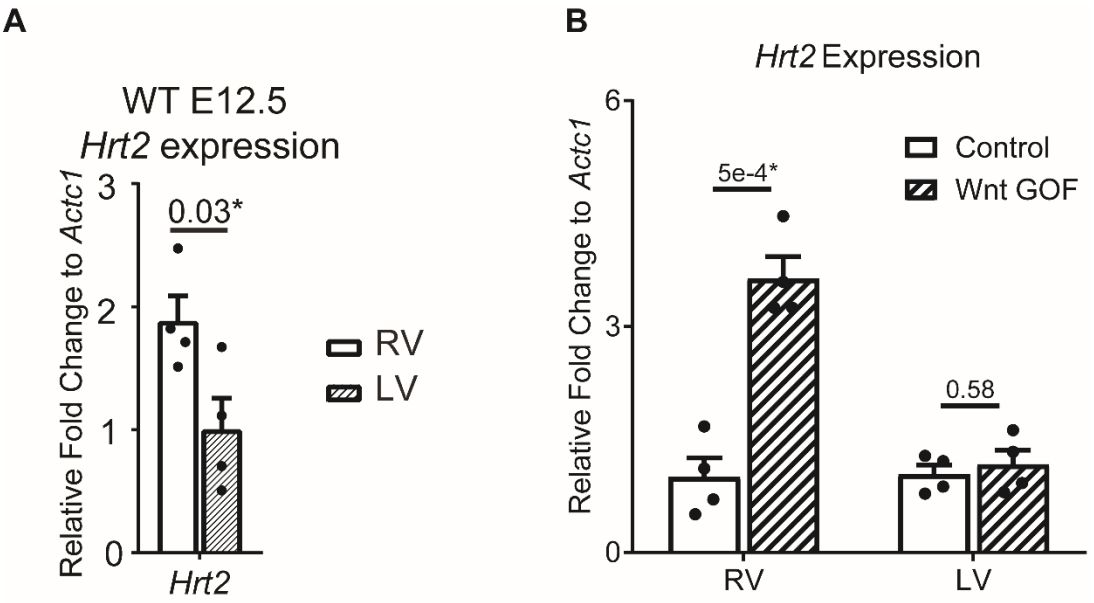
Supplemental Figure 4. Principal Component analysis of Wnt gain-of-function RNA-sequencing transcript results. All 6 control and 5 of 6 Wnt GOF samples cluster closely together with clear separation between control and Wnt GOF samples.



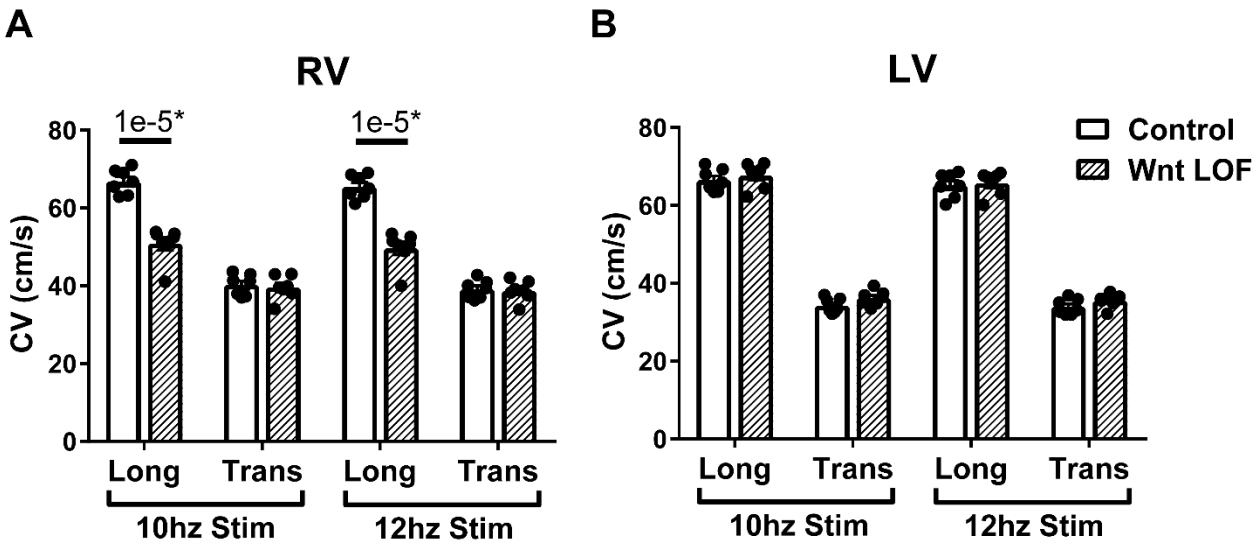
Supplemental Figure 5. ENCODE data of mouse heart H3K4me1 and H3K27ac seq shows peaks near *Hrt2* enhancers. Mouse heart ChIP-seq for H3K4me1 and H3K27ac from ENCODE in embryonic day 10.5 and 12.5 (E10.5, E12.5), postnatal day 0 (P0), and adult (8 Weeks) show enrichment peaks at all three *Hrt2* enhancer sequences at E10.5, E12.5 and PO compared to control, with decreased amplitude of the peaks in adult hearts. This is consistent with the known decrease in *Hrt2* expression postnatally.



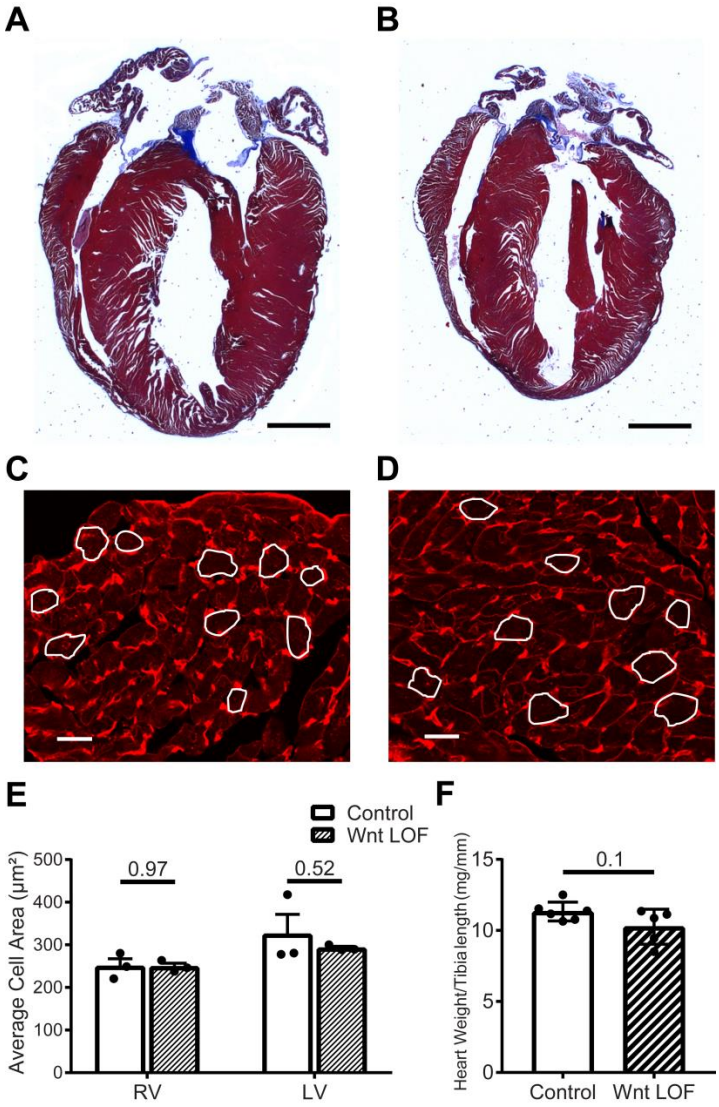
Supplemental Figure 6. *Hrt2* enhancer 3 is largely conserved between mice and humans. Mouse reference sequence (Mm9) of *Hrt2* enhancer 3 compared to human reference sequence shows overall 82% sequence conservation. A portion of enhancer 3 is shown here, with 3 conserved GATA binding sites between mouse and human (yellow). The murine Wnt-responsive element is also conserved, while there are 3 additional Wnt-responsive elements in the human sequence (green).



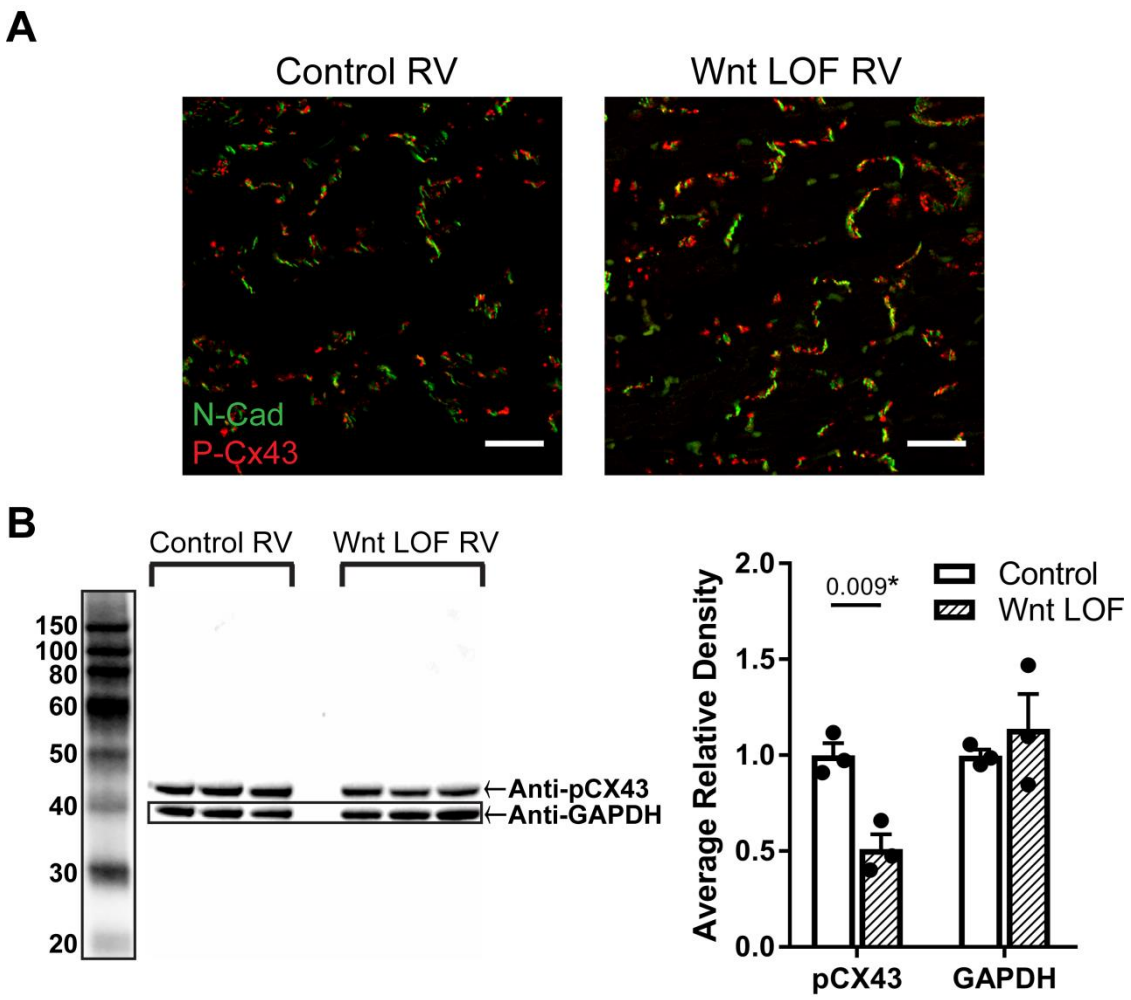
Supplemental Figure 7. *Hrt2* expression in embryonic wild type and in adult Wnt gain-of-function ventricles. (**A**) qRT-PCR of WT E12.5 primitive right and left ventricles (n= 4 each) shows 1.8-fold higher *Hrt2* expression in embryonic RV compared to LV. (**B**) Comparison of RV and LV of control (*Mlc2v*$^{Cre/+}$) versus Wnt GOF (*Mlc2v*$^{Cre/+}$; *Ctnnb1*$^{fl(ex3)/+}$) shows increased *Hrt2* expression in RV only (n=4 each group). An equal variance Student's t test was used for comparisons. *P<0.05 was considered statistically significant.



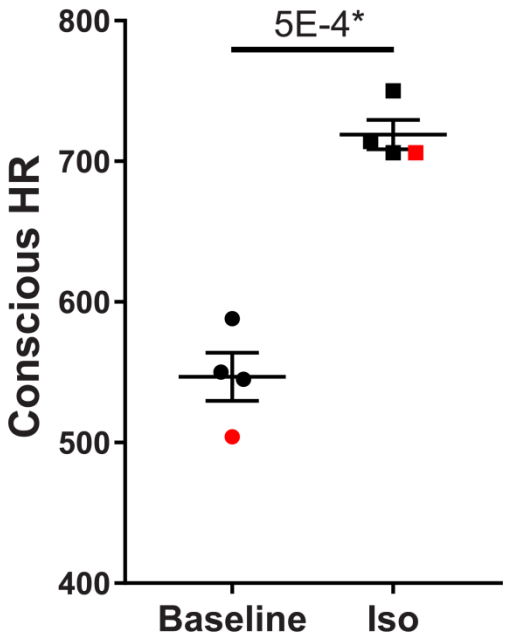
Supplemental Figure 8. Conduction velocity is slower in Wnt loss-of-function right ventricles when paced at physiologic rates. Longitudinal and transverse conduction velocities from control and Wnt LOF hearts (n=7 each genotype) show significantly reduced RV longitudinal CV compared with control RV (**A**) at 10Hz and 12Hz stimulation, while both longitudinal and transverse CV is unchanged in the LV (**B**). An equal variance Student's t test was used for comparisons, *P<0.05 were considered statistically significant. Long = longitudinal, Trans = transverse.



Supplemental Figure 9. Structural determinants of conduction velocity are not changed in Wnt loss-of-function hearts. Representative images of Masson's Trichrome stain of control (**A**) and littermate Wnt LOF (**B**) heart shows no gross structural heart defects (n=3 each genotype). Scale = 1000 µm. Wheat germ agglutinin staining of cell membranes shows no gross difference in transverse myocyte cross-sectional area between control (**C**) and littermate Wnt LOF (**D**) RV cardiomyocytes. Representative cells used for quantification are outlined in white. Only ventricular myocytes sectioned perpendicularly, as evidence by circular morphology, were included in the analysis. Scale = 25 µm. (**E**) Quantification of RV and LV myocyte cross-sectional area shows no significant differences between control and Wnt LOF hearts (n=3 each genotype). Each data point represents the average area of more than 100 cells from the same heart. (**F**) Wnt LOF mice are smaller when compared with littermate control mice of the same age, however, the heart weight to tibia length ratio is not significantly different (n=6 each genotype). An equal variance Student's t test was used for comparisons. *P<0.05 was considered statistically significant.



Supplemental Figure 10. Levels and localization of phospho-Connexin 43 (pCx43) are unchanged in Wnt loss-of-function RV. (**A**) Representative image of immunostaining of pCx43 (red) and N-Cadherin (green) shows strong co-localization at the intercalated disc in both control (*Ctnnb1*$^{flox/+}$) and littermate Wnt LOF (*Mlc2v*$^{Cre/+}$*; Ctnnb1*$^{dm/flox}$) RV without evidence of lateralization (n=3 each genotype). Scale = 20 μm**.** (**B**) Western blot comparing levels of pCx43 (~42 kDa) in control RV and littermate Wnt LOF RV (n= 3 each genotype)**.** GAPDH is used for normalization, and quantification of protein levels based on band density shows a 50% reduction of pCx43 in Wnt LOF RV, similar to the reduction of total Cx43 levels. An equal variance Student's t test was used for comparisons. *P<0.05 was considered statistically significant.



Supplemental Figure 11. Heart rate as measured by telemetry is increased following isoproterenol injection. Heart rate recorded via telemetry in control (*Ctnnb1*$^{flox/+}$, n= 1, red) and Wnt LOF (*Mlc2v*$^{Cre/+}$*; Ctnnb1*$^{dm/flox}$, n= 3, black) mice shows increased HR following intraperitoneal isoproterenol injection. However, no spontaneous arrhythmias were observed in either condition. A paired t test was used to determine significance between baseline and isoproterenol HR. *P<0.05 was considered statistically significant.

Supplemental Tables

Supplemental Table 1. List of transcripts changed in both Wnt LOF RV and LV

	RV		LV	
Gene Name	Fold Change	p-value	Fold Change	p-value
1110008P14Rik	1.22	0.0378	-1.35	0.006
A930005H10Rik	-1.54	0.0201	-1.81	0.0057
Abra	-1.30	0.0374	-1.52	0.0033
Actc1	1.17	0.0352	-1.23	0.0087
Adcy4	-1.25	0.0391	-1.31	0.0171
Adhfe1	-1.32	0.0063	-1.25	0.0248
Alas2	-1.22	0.0394	1.24	0.0397
Amz1	-1.50	0.0189	2.09	0.0008
Ano4	1.43	0.0010	1.38	0.0029
Aqp1	-1.41	0.0008	-1.22	0.0266
Auts2	1.19	0.0318	1.26	0.0067
B330016D10Rik	1.31	0.0226	1.29	0.035
Bcl11b	1.54	0.0037	1.41	0.0239
Bicd1	1.25	0.0107	1.18	0.0461
Brca2	1.23	0.0482	1.28	0.0231
C920006O11Rik	2.79	0.0018	2.07	0.0137
Cnih4	1.38	0.0066	1.26	0.0399
csf1	1.37	0.0010	1.26	0.01
Decr2	-1.18	0.0148	-1.14	0.0422
Dixdc1	1.32	0.0214	1.29	0.0391
Dnajc5	1.51	0.0250	1.44	0.0452
Dnm3os	1.26	0.0319	1.25	0.0392
Dysf	1.12	0.0319	1.17	0.0072
Ednrb	-1.29	0.0283	-1.59	0.0007
Fabp5	-1.57	0.0025	-1.54	0.0039
Fam220a	-1.13	0.0454	-1.17	0.0147
Fasn	1.15	0.0290	1.16	0.0232
G0s2	-1.31	0.0371	-1.35	0.0302
Gm15334	1.30	0.0347	1.37	0.0123
Gm15446	2.17	0.0018	1.56	0.0361
Gm17203	1.41	0.0480	1.69	0.0061
Gm24041	-1.47	0.0219	1.50	0.0211
Gm28979	1.40	0.0190	1.34	0.0438
Gm6612	-5.38	0.0218	-4.50	0.0414
Heatr5b	-1.31	0.0028	-1.18	0.05
Hsd17b7	6.76	0.0015	4.91	0.0059
Hsp90aa1	-1.30	0.0250	-1.35	0.0101
Hspa12a	1.36	0.0021	1.34	0.0029
Ky	1.26	0.0086	1.18	0.045

Lgi2	2.55	0.0022	3.03	0.0008
Lgr6	1.48	0.0147	1.38	0.0379
Lrif1	1.16	0.0298	-1.14	0.0477
Ly6e	1.24	0.0097	-1.24	0.0109
Ly75	1.32	0.0439	1.44	0.0153
Maf	-1.22	0.0163	1.28	0.0135
Mical2	-1.18	0.0334	-1.20	0.024
Muc5b	-1.59	0.0472	2.28	0.0032
Mylk4	1.22	0.0356	1.23	0.0279
Mzt1	1.20	0.0432	1.24	0.0232
Nfia	1.22	0.0120	1.22	0.0132
Nxpe4	17.36	0.0152	5.13	0.0429
Olfml2b	-1.40	0.0014	-1.40	0.0018
P4ha1	-1.25	0.0412	-1.30	0.0209
Pcp4l1	1.31	0.0079	1.33	0.0072
Pdgfc	2.09	0.0017	1.46	0.0484
Plb1	1.49	0.0314	1.50	0.0378
Plin2	-1.33	0.0008	-1.23	0.0083
Plscr2	1.14	0.0485	1.15	0.0356
Ppil1	-1.48	0.0208	-1.61	0.0098
Ppp1r16b	-1.18	0.0295	-1.29	0.0021
Prox1	1.21	0.0140	1.20	0.0218
Rab37	2.06	0.0149	1.81	0.0266
Rab6b	6.19	0.0035	3.39	0.0297
Reps2	1.25	0.0471	1.35	0.0118
Rgs4	-1.34	0.0444	-1.37	0.0334
Ripor2	1.34	0.0198	1.49	0.0036
Rnf150	1.16	0.0192	1.15	0.0241
Rpl26	2.20	0.0304	3.07	0.0061
Rps2-ps13	12.79	0.0158	15.61	0.0277
Rtca	-1.25	0.0378	-1.28	0.0295
Rybp	1.32	0.0372	-1.35	0.0268
Serpine1	-1.41	0.0016	-1.39	0.0024
Setbp1	1.24	0.0500	1.26	0.0397
Slc12a9	1.38	0.0170	1.41	0.0097
Slc38a1	1.56	0.0176	1.45	0.0371
Smoc2	-1.18	0.0242	-1.16	0.0459
Sqstm1	-1.17	0.0307	-1.21	0.0139
Tjap1	-1.19	0.0412	-1.22	0.0236
Tuba1a	-1.31	0.0447	-1.32	0.0421
Tubb2b	-2.00	0.0146	-1.84	0.0276
Uba52	-1.29	0.0266	-1.33	0.0248
Zfp608	-1.15	0.0496	-1.16	0.0466

Supplemental Table 2. List of Wnt-mediated genes from RNA-sequencing of Wnt GOF embryonic ventricles

Gene Name	Fold Change	p-value
1700080G11Rik	5.95	0.0422
Adamts3	-1.54	0.0494
Angpt1	1.56	0.0343
Aplp2	1.64	0.0378
Bco2	7.89	0.0289
Ccnd2	1.42	0.0378
Cdh6	2.38	0.0286
Col23a1	1.47	0.0378
Creg2	5.76	0.0378
Cyp26b1	5.97	0.0378
Dkk4	10.94	0.0098
Eln	-3.67	0.0378
Fam213b	-1.51	0.0472
Fibcd1	17.19	0.0343
Gm26984	5.65	0.0422
Gm29521	11.06	0.0104
Gnb1	-1.51	0.0343
Hrt2	1.34	0.0343
Hsbp1l1	2.18	0.0378
Hspa2	2.07	0.0098
Jakmip3	6.93	0.0378
Lgi1	5.89	0.0378
Limch1	1.33	0.0472
Notumos	6.36	0.0343
Nupr1	-2.98	0.0022
Rab33a	2.87	0.0343
Rgs5	-6.37	0.0209
Rnf43	3.96	0.0453
Rxfp1	5.07	0.0378
Scn4b	5.48	0.0422
Skap1	5.49	0.0343
Slc13a4	3.48	0.0422
Smoc2	-2.01	0.0286
Sp5	16.74	0.0286
Tenm2	1.84	0.0289
Trpm1	6.32	0.0289

Supplemental Table 3. Microelectrode Recordings

	Control RV n=5	Wnt LOF RV n=5	Control LV n=5	Wnt LOF LV n=5
RMP, mV	-79.6± 0.5	-78.7 ± 0.6	-79.0 ± 0.4	-78.6 ± 0.6
APA, mV	87.6 ± 0.9	90.1 ± 1.0	81.2 ± 1.4	83.7 ± 1.5
dV_m/dt_{max}, V/sec	230.0 ± 6.4	250.5 ± 4.3*	180.6 ± 9.2^	173.8 ± 7.5
APD_{90}, msec	48.9 ± 5.1	43.7 ± 2.7	65.2 ± 5.2^	57.3 ± 2.6

Action potential parameters from 8-10 cells are averaged to yield a value per mouse. The average result from n=5 mice per genotype are expressed as mean ± SEM. Control indicates *Ctnnb1*$^{flox/+}$ and Wnt LOF indicates *Mlc2v*$^{Cre/+}$*; Ctnnb1*$^{dm/flox}$ experimental groups. Statistics were performed using a one-way ANOVA followed by a Tukey's multiple comparisons test. Values of $P<0.05$ were considered statistically significant in comparison between control RV versus control LV (^*P*<0.05), as well as Wnt LOF vs. control mice (*P<0.05). RMP = resting membrane potential; APA = action potential amplitude; dV_m/dt_{max} = maximal upstroke velocity; APD_{90} = duration of the action potential at 90% repolarization.

Supplemental Video Legends

Supplemental Video 1. Phase movie of a Wnt LOF heart in sinus rhythm during an optical mapping experiment with RA and RV in view. The earliest ventricular breakthrough is near the apex of the RV.

Supplemental Video 2. Phase movie of the same Wnt LOF heart from Supplemental Video 1 during ventricular tachycardia induced by physiologic RV stimulation as viewed from the anterior surface. A line of functional block with reentry can be seen with alternating beats near the septum.