Supplemental Table 1. Echocardiography

	Control (n=4)	*Mlc2v$^{cre/+}$; DNMAML* (n=4)
LVIDd, mm	3.9±0.3	4.3±0.3
LVIDs, mm	2.6±0.4	2.9±0.2
IVSd, mm	0.72±0.06	0.75±0.1
LVPWd, mm	0.72±0.06	0.77±0.11
FS, %	33±6	33±1
EF, %	61±10	62±2

Mean values ± SD. p values not statistically significant.

LVIDd=left ventricular diameter at end diastole, LVIDs=left ventricular diameter at end-systole, IVSd=interventricular septal wall thickness at end diastole, LVPWd=left ventricular posterior wall thickness at end diastole, FS=fractional shortening, EF=ejection fraction

Supplemental Table 2. Notch-1 deficiency disrupts AV nodal delay

	Control (n=12)	*Mlc2v*$^{cre/+}$*; Notch1*$^{flox/flox}$ (n=12)
HR (bpm)	491±58	520±71
PR (ms)	39.4±3.2	36.8±2.4*

Mean values ± SD. *p<0.03 for control *Notch1*$^{flox/+}$ and *Notch1*$^{flox/flox}$ versus *Mlc2v*$^{cre/+}$*; Notch1*$^{flox/flox}$.

Supplemental Table 3. Echocardiography

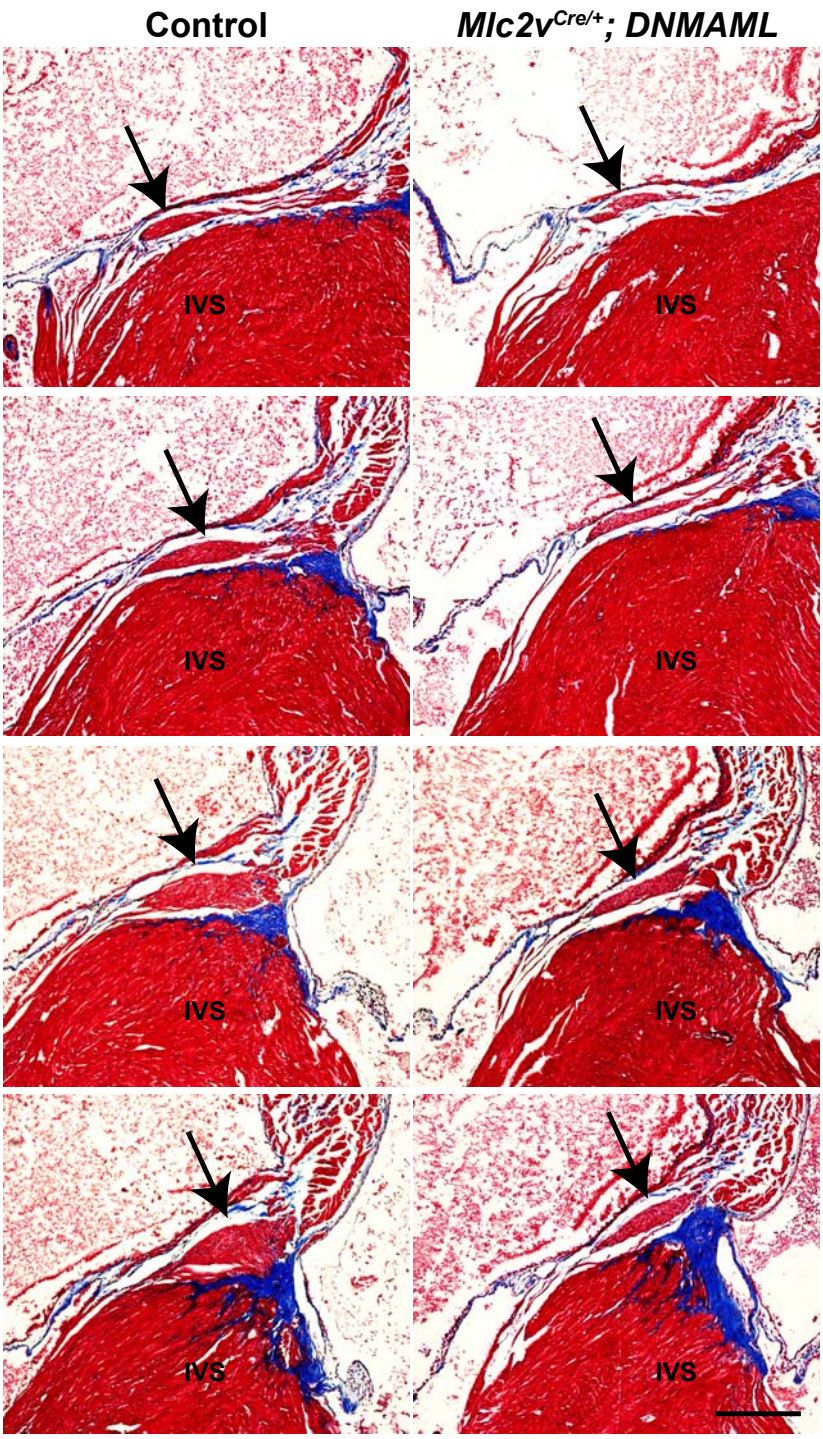
	Control (n=3)	*Mlc2v$^{cre/+}$; NICD* (n=3)
LVIDd, mm	4.6±0.1	4.2±0.3
LVIDs, mm	2.9±1	2.7±0.2
IVSd, mm	0.76±0.03	0.65±0.03*
LVPWd, mm	0.73±0.05	0.65±0.03
FS, %	37±2	35±2
EF, %	67±2	65±3

Mean values ± SD. *P<0.05 for control versus *Mlc2v$^{cre/+}$; NICD.*

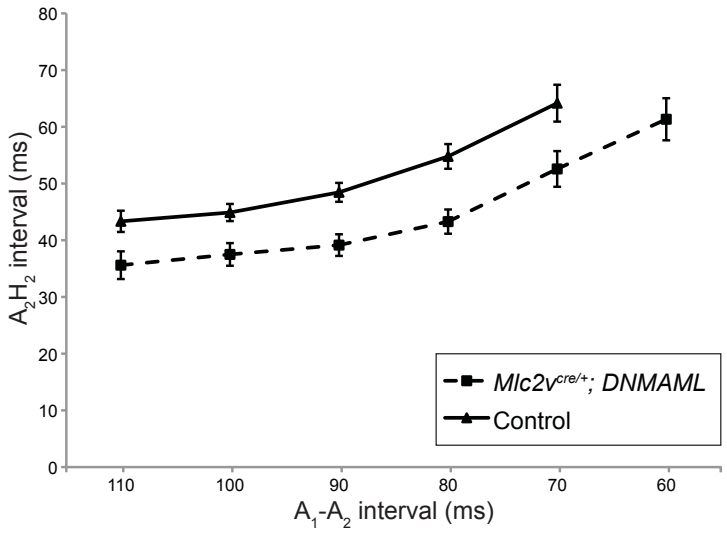
LVIDd=left ventricular diameter at end diastole, LVIDs=left ventricular diameter at end-systole, IVSd=interventricular septal wall thickness at end diastole, LVPWd=left ventricular posterior wall thickness at end diastole, FS=fractional shortening, EF=ejection fraction

Supplemental Results:

EKGs were performed using two different anesthetic agents to minimize the chances that the observed effects on AV conduction are secondary to the anesthetic. The data using pentobarbital is presented in Table 1 of the manuscript. Pentobarbital is known to suppress the heart rate, prolong the PR interval, QRS and QT intervals in mice (Zeller, A., et al. *Molecular Pharmacology*, 2007, 71(3):852-859). However, inhaled isoflurane has been shown to have minimal effects on murine heart rate and EKG intervals during the first several minutes of application, as was done in our studies (Zeller, A. et al. *BMC Pharmacology* 2007, 7:2). Using inhaled isoflurane, the heart rate was 524±82 beats/min in n=9 *Mlc2v*$^{cre/+}$*; DNMAML* mice versus 514±74 beats/min in n=12 *Mlc2v*$^{+/+}$*; DNMAML* mice ($p<0.78$). Although the heart rate was no longer significantly shorter with isoflurane, the PR interval did remain significantly shorter in Notch inactivated mice (35.7±3.0 ms in n=9 *Mlc2v*$^{cre/+}$*; DNMAML* mice versus 38.9±3.2 ms in n=12 *Mlc2v*$^{+/+}$*; DNMAML* mice, $p<0.03$).

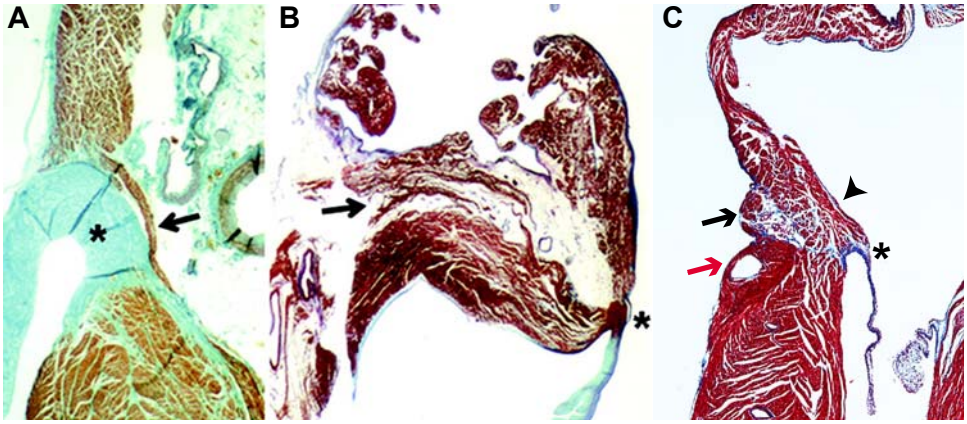


Supplemental Figure 1. Notch inhibited mice have a smaller AV node when compared with control. Representative trichrome-stained images from similar regions of a control (left) and *Mlc2v*$^{Cre/+}$*; DNMAML* (right) heart. Images are shown from posterior to anterior with respect to anatomic location , and the region of the AV node is denoted with a black arrow. Scale bar = 100 μm. Control heart is *Mlc2v*$^{+/+}$*; DNMAML.* IVS=interventricular septum

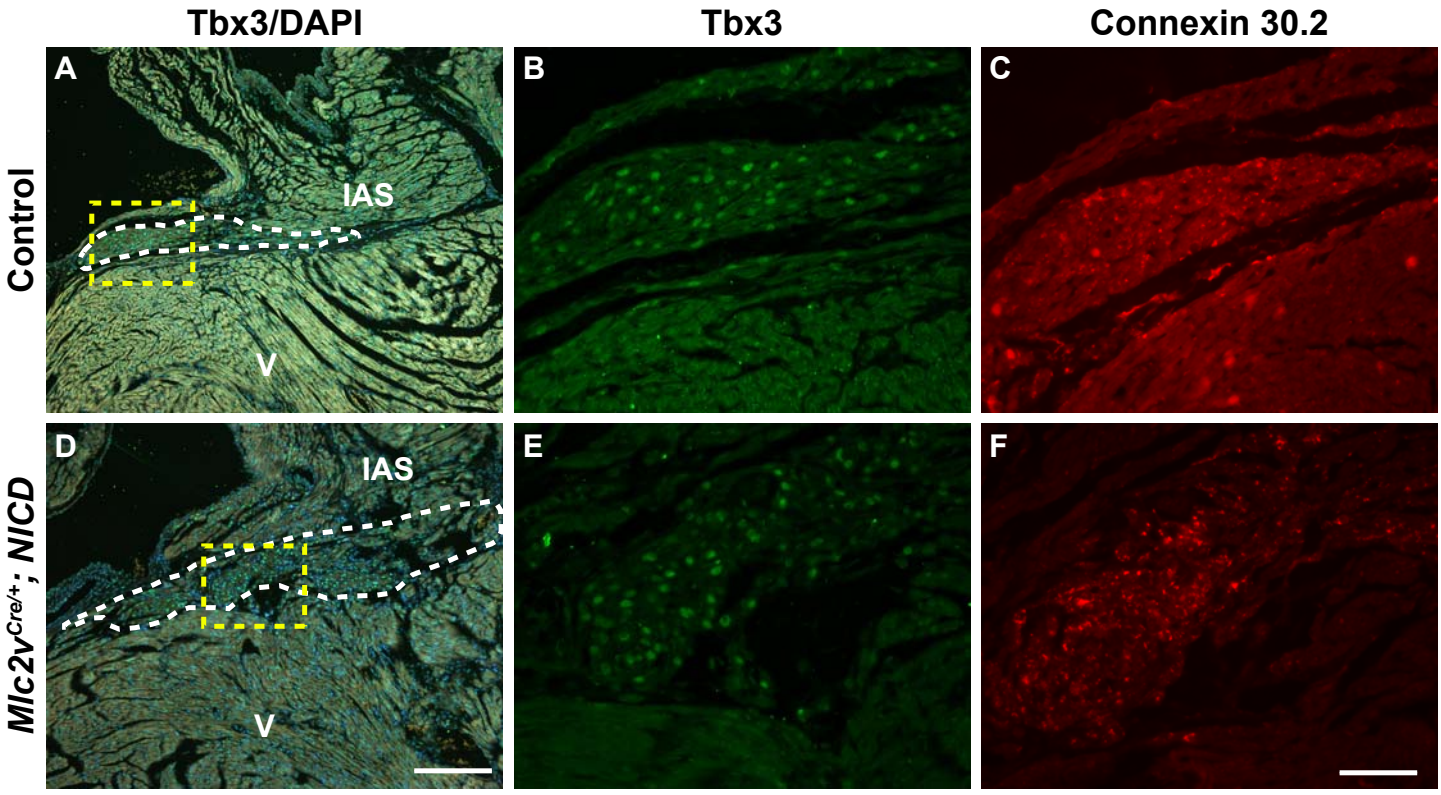


Supplemental Figure 2: Notch inactivated mice have abnormal AV nodal function.

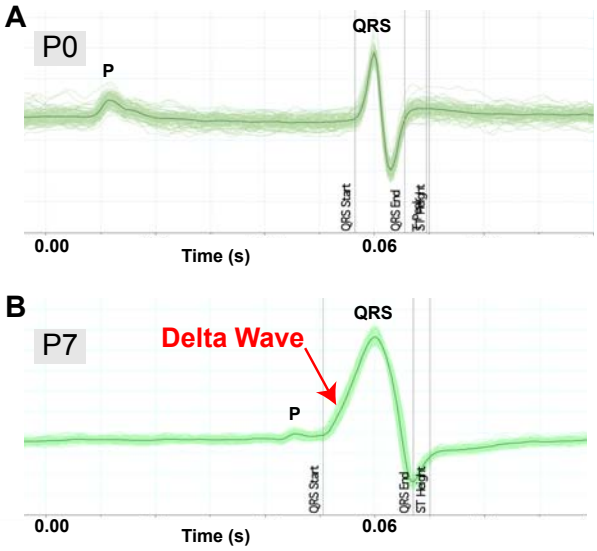
Programmed electrical stimulation was performed with an atrial drive train of 120 ms (A_1) and increasingly premature atrial stimulation (A_2). The effect of premature atrial stimulation on the subsequent AH interval (A_2-H_2) was measured. At every A_1-A_2 interval tested, *Mlc2v$^{Cre/+}$; DNMAML* mice have a shorter A_2-H_2 interval than controls. In addition, the prolongation of the A_2-H_2 interval in *Mlc2v$^{Cre/+}$; DNMAML* mice is more consistent with decremental conduction through AV nodal tissue and not accessory pathway tissue. Control mice are *Mlc2v$^{+/+}$; DNMAML.*



Supplemental Figure 3. Notch activated mice have accessory pathways reminiscent of human accessory pathways.
Histologic sections from human WPW hearts (A,B reproduced with permission from Wolters Kluwer Health, Ho, S.Y. Accessory atrioventricular pathways: getting to the origins. *Circulation.* 2008;117(12):1502-1504) and a *Mlc2v$^{Cre/+}$; NICD* mouse heart (C) stained with Masson's Trichrome (myocardium in red). Panel A shows an epicardial accessory pathway skirting a well-formed mitral annulus. Panel B shows a right-sided accessory pathway formed by a muscularized right ventricular vein. Panel C shows an epicardial accessory pathway in a Notch activated mouse heart skirting fatty tissue in the AV junction (black arrow). The accessory pathway arises from a region of the right ventricle with a muscularized vein (red arrow). The arrowhead points to myocardial tissue along the atrial surface of the tricuspid valve. *valvular annulus



Supplemental Figure 4. **Expanded AV node in Notch activated hearts.**
A,D. The dashed white line delineates theTbx3-positive, connexin 30.2-positive, connexin 40-negative (not shown) AV nodal region, which is expanded in the *Mlc2v$^{Cre/+}$; NICD* heart (D) when compared with control (A). Immunohistochemistry of the region denoted by the yellow box is shown at higher magnification for Tbx3 in green (B,E) and for connexin 30.2 in red on a serial slide (C,F). Control genotype is *Mlc2v$^{+/+}$; NICD.* Scale bar A,D = 200 μm. Scale bar B,C,E,F =100 μm. V=Ventricle, IAS= interatrial septum



Supplemental Figure 5. Progressive Development of Preexcitation. Signal-averaged EKG (average of 100 beats) recorded from the same *Mlc2v*$^{Cre/+}$*; NICD* mouse at birth (A) and at one week of life (B). Note the progressive post-natal development of preexcitation as evidenced by the development of a shortened PR interval, widened QRS complex, and presence of a Delta wave.