SUPPLEMENTAL MATERIAL

Supplemental Methods

qPCR Primers

ß-actin F 5'-CTGCCTGACGGCCAAGTCATCAC–3'
ß-actin R 5'-GTCAACGTCACACTTCATGATGG–3'

Cntn2 F 5'-CATGTCTTCAGCCACTGACC–3'
Cntn2 R 5'-TGGCCTTGTCCTGGGTTAT–3'

Cx30.2 F 5'–TGATCATGCTGATCTTCCGCATCC–3'
Cx30.2 R 5'–GCTGCAACGTGTTACACACGAACT–3'

Cx43 F 5'-ACAAGGTCCAAGCCTACTCCA–3'
Cx43 R 5'-CCCCAGGAGCAGGATTCTGA-3'

Cx40 F 5'-GAGGCCCACGGAGAAGAATG–3'
Cx40 R 5'–TGGTAGAGTTCAGCCAGGCT-3'

HCN1 F 5'-GTCTTTACTCCCTTTCGGTGG-3'
HCN1 R 5'-ATCCTTCTGGAACTTCTGCAG-3'

HCN2 F 5'-CTTCACCAAGATCCTCAGTCTG-3'
HCN2 R 5'-TGATCAGGTTACAGATGCGC-3'

HCN4 F 5'-TTGACTCGGAGGTCTACAAAAC-3'
HCN4 R 5'-CAGGTCATAGGTCATGTGGAAG-3'

Nkx2.5 F 5'–CCACTCTCTGCTACCCACCT-3'
Nkx2.5 R 5'–CCAGGTTCAGGATGTCTTTGA-3'

Scn5a F 5'–GAAGAAGCTGGGCTCCAAGA-3'
Scn5a R 5'–CATCGAAGGCCTGCTTGGTC-3'

Hop F 5'-TTCAACAAGGTCAACAAGCACCCG-3'
Hop R 5'-CCAGGCGCTGCTTAAACCATTTCT-3'

Tbx2 F 5'–TAAACGCATGTACATCCACCCGGA-3'
Tbx2 R 5'–GCTTCAAGATGTCATTGGCTCGCA-3'

Tbx3 F 5'–GAACCCGAAGAAGACGTAGAAG-3'
Tbx3 R 5'–AGAGCACCTCACTTTAAACGG-3'

Tbx5 F 5'–ACCTGGACCCGTTTGGACACATTA-3'

Tbx5 R 3’–ACGCAGTGTTCTTTGAACCGAACC-3’

Hrt1 F 5’–GAAGCGCCGACGAGACCGAATCAA-3’
Hrt1 R 5’-CAGGGCGTGCGCGTCAAAATAACC-3’

Hrt2 F 5′-CGACGTGGGGAGCGAGAACAAT-3’
Hrt2 R 5′-GGCAAGAGCATGGGCATCAAAGTA-3’

Hrt3 F 5’–GGTCCCCACTGCCTTTGAGA-3’
Hrt3 R 5’–TAGCTGACTGCTCAGGGAAGGCAA-3’

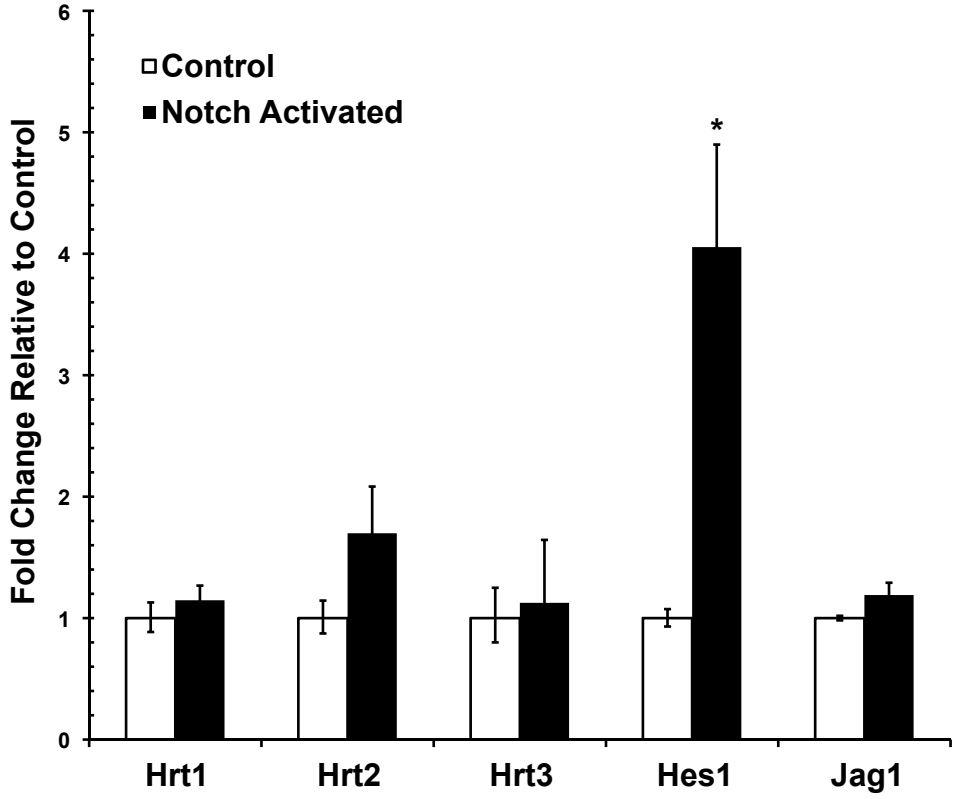
Jag1 F 5’–CTACTGTGATTGCCTTCCTGG-3’
Jag1 R 5’–GTGGACAGATACAGCGATAACC-3’

Hes1 F 5’–AAAGCCTATCATGGAGAAGAGGCG-3’
Hes1 R 5’–GGAATGCCGGGAGCTATCTTTCTT-3’

Deltex1 F 5’-ATCAGTTCCGGCAAGACAC-3’
Deltex1 R 5’-TGATGCAGATGTCCATGTCG-3’

Bmp2 F 5’–TGTGGGCCCTCATAAAGAAGCAGA–3’
Bmp2 R 5’–AGCAAGCTGACAGGTCAGAGAACA–3’

Actc1 F 5’ – GACCTCACTGACTACCTCATG–3’
Actc1 R 5’ – TCTCGTTCTCAAAATCCAGGG–3’

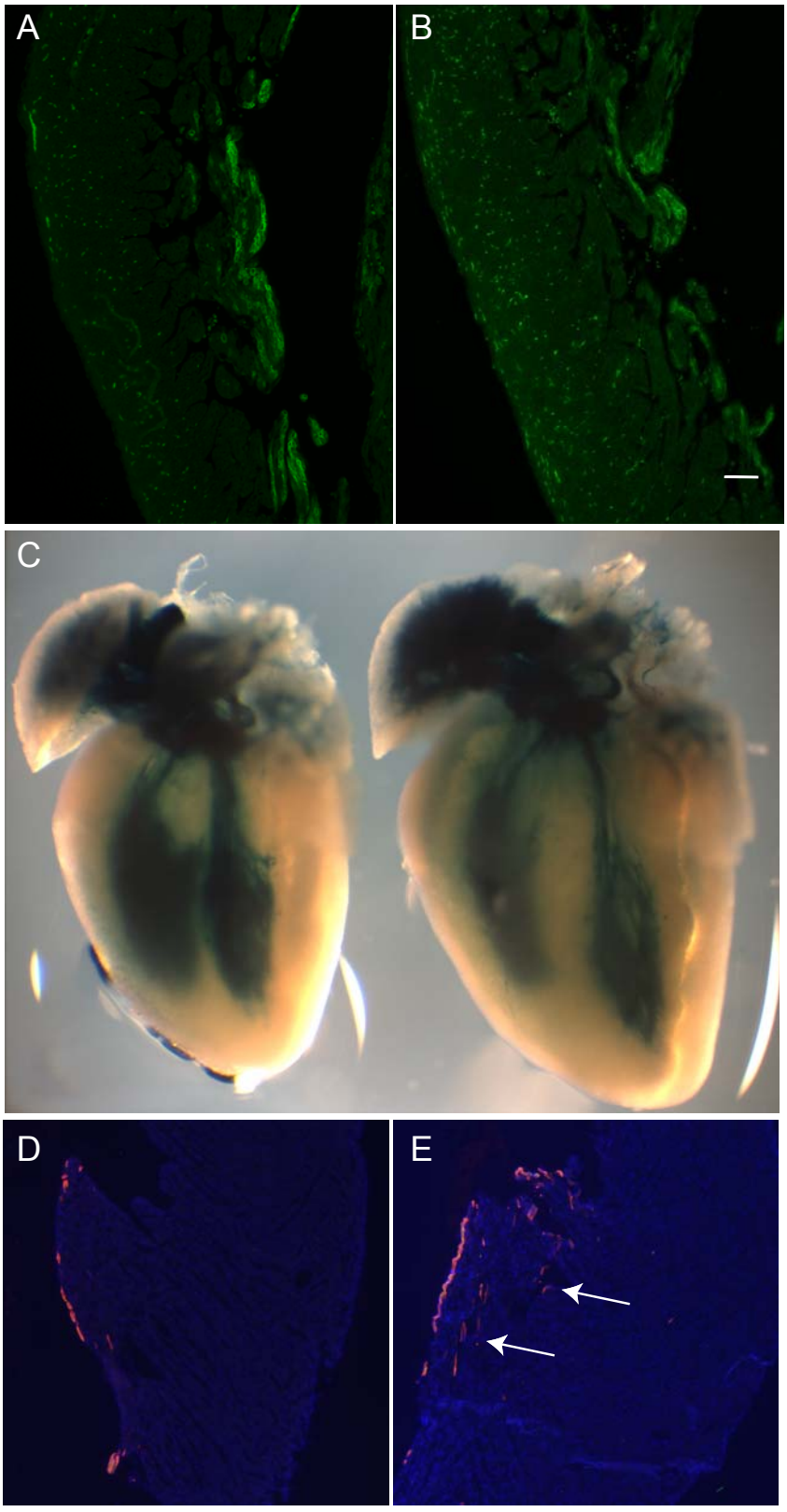


Supplemental Figure 1

Supplemental Figures:

Supplemental Figure 1. Activation of Distinct Notch Targets in Adult Myocardium.

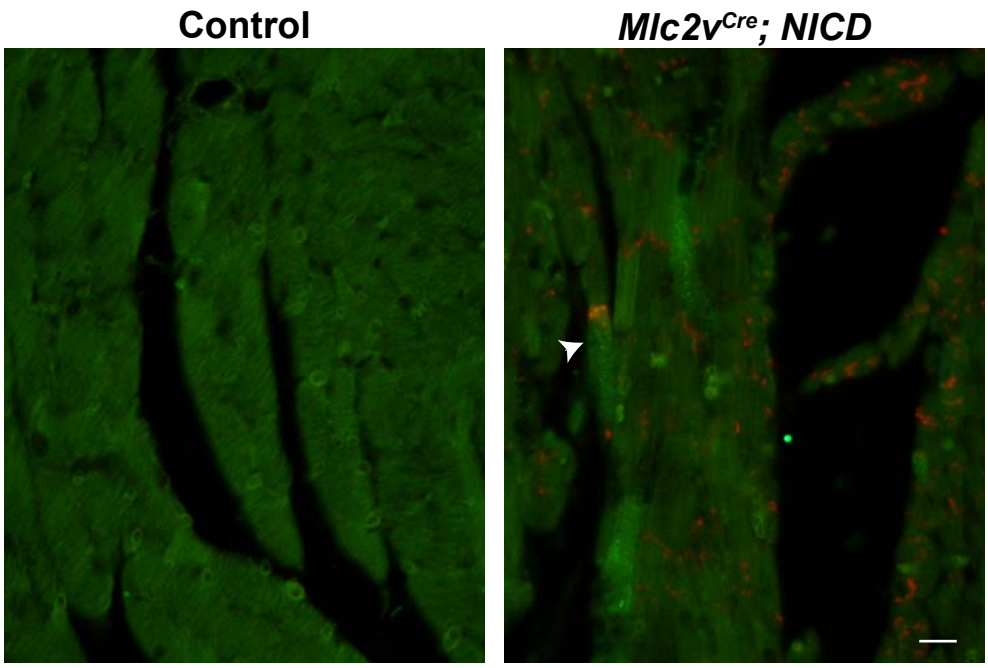
RT-qPCR analysis of Notch target gene expression in adult ventricular samples from Notch activated hearts versus control hearts. *Hes1* was significantly up-regulated, while other known canonical Notch targets were unaffected in adult ventricular tissue. Control mice are *NICD* littermates. n=3 each genotype. Data are expressed as mean ± SEM. Group comparison was performed using a Student's unpaired 2-tailed *t*-test. *$p<0.05$



Supplemental Figure 2

Supplemental Figure 2. Progressive Conduction System Mispatterning in Notch Activated Hearts

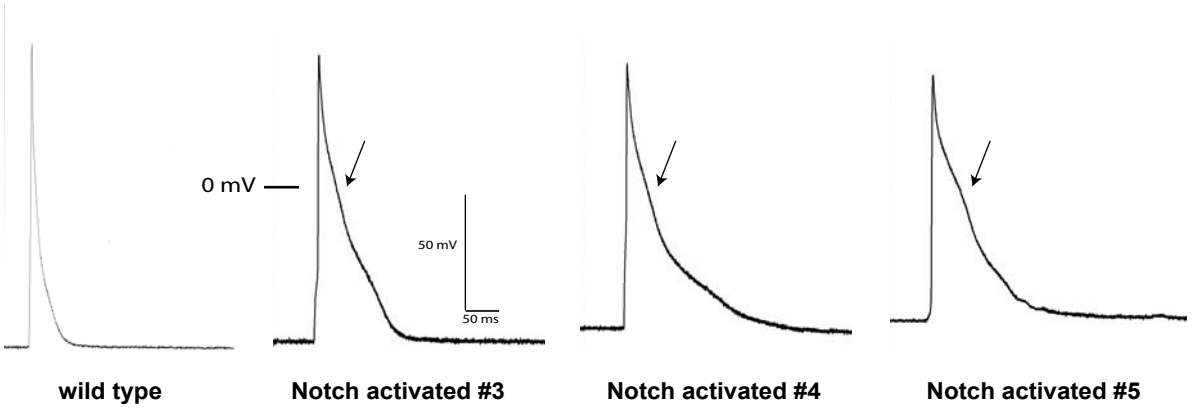
Immunohistochemistry for Cntn2 (green) in a control (A) and a Notch activated (B) newborn heart in a region of the right ventricular free wall. In contrast to Cntn2 up-regulation at later postnatal stages (Figure 1), at birth there is no apparent difference in Cntn2 expression. Similarly, conduction system patterning as assessed by *CCS-LacZ* expression is normal at birth in Notch activated hearts (C, right) when compared with control (C, left). Immunohistochemistry for EGFP (red) in *Cntn2-EGFP* (D) versus *Mlc2v$^{Cre/+}$; Cntn2-EGFP: NICD* (E) mice at four weeks of age demonstrates ectopic expression of this conduction system marker along the left ventricular free wall and blurring of the normally sharp boundary between conduction and chamber myocardium (arrows). Scale bar = 100 μM



Supplemental Figure 3

Supplemental Figure 3. Colocalization of Cntn2 and Cx40 in Ectopic CCS Tissue

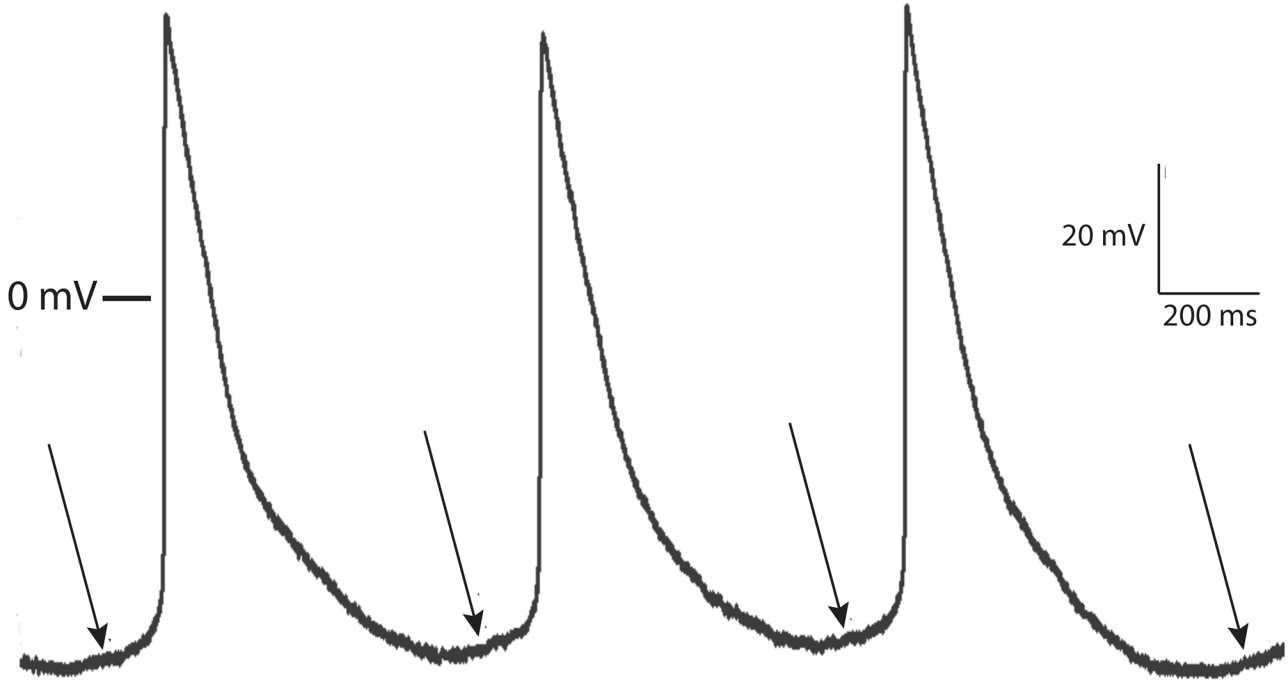
Immunohistochemistry for Cntn2 (green) and Cx40 (red) demonstrates ectopic expression of both Purkinje markers within the ventricular region in *Mlc2v$^{Cre/+}$; NICD* mice when compared with a similar region in control hearts. Some cells ectopically express both Cntn2 and Cx40 (white arrowhead), while others have not fully reprogrammed and express either Cntn2 or Cx40. Scale bar = 20 μM.



Supplemental Figure 4

Supplemental Figure 4. Notch Activation Leads to Purkinje-like Action Potentials

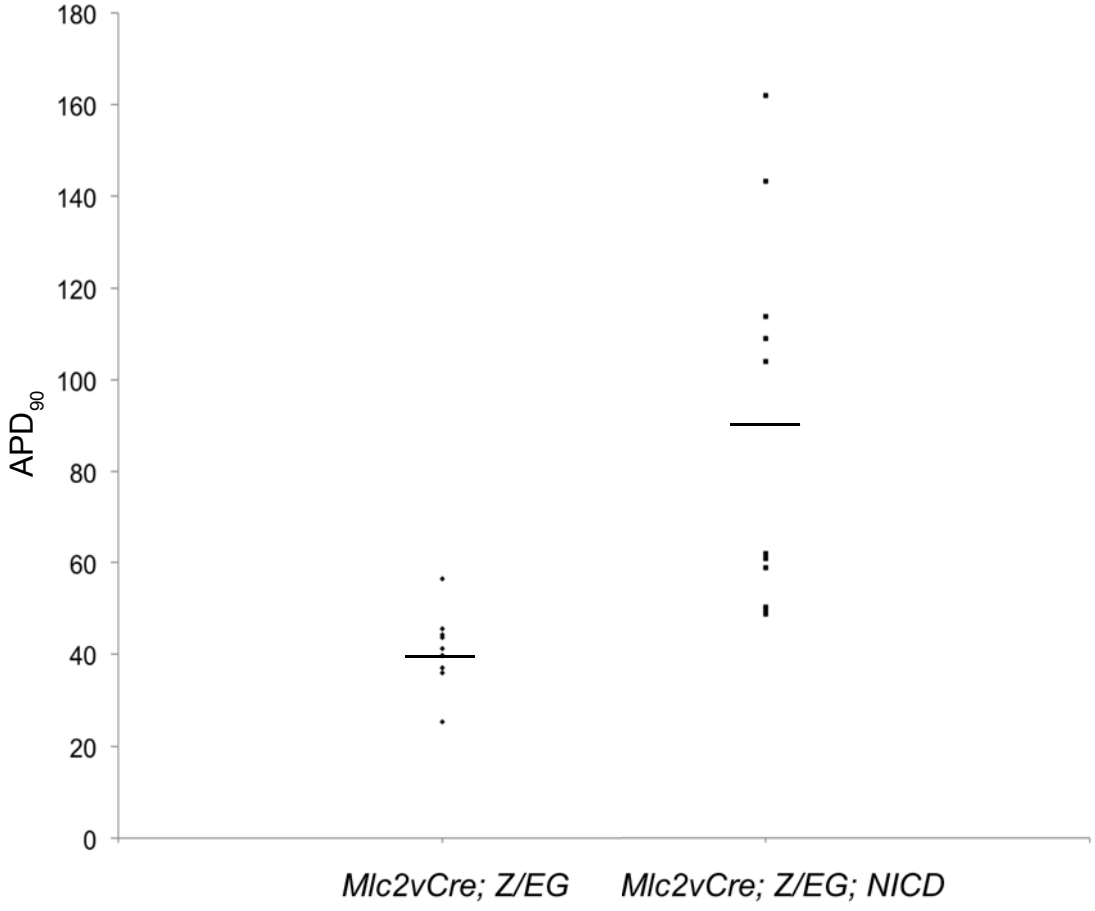
When compared with wild type ventricular cardiomyocytes (left), most Notch activated cells have a prolonged action potential with a characteristic Purkinje-like dome in phase 2 (arrows). See also Figure 2B.



Supplemental Figure 5

Supplemental Figure 5. Notch Activation Induces Spontaneous Action Potentials with Phase 4 Depolarization

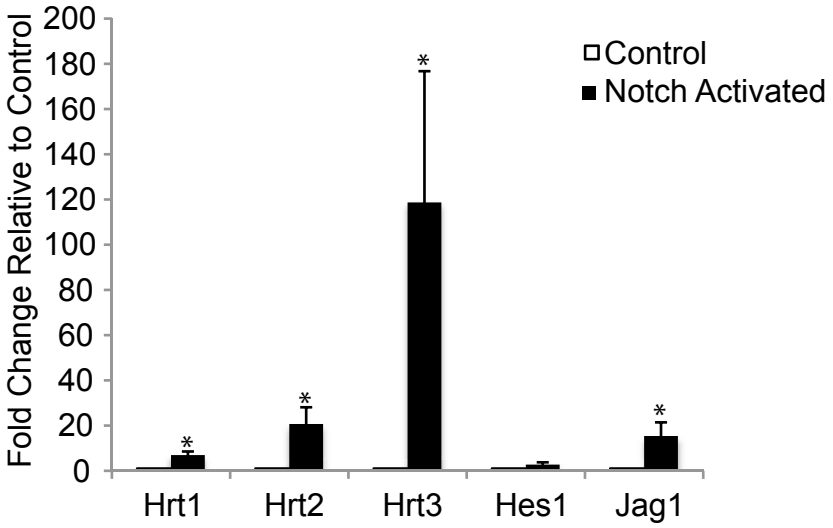
Shown are three consecutive spontaneous depolarizations from a Notch activated cell. Note that each action potential is preceded by phase 4 depolarization (arrows), and that the action potentials are prolonged with a Purkinje-like dome in phase 2.

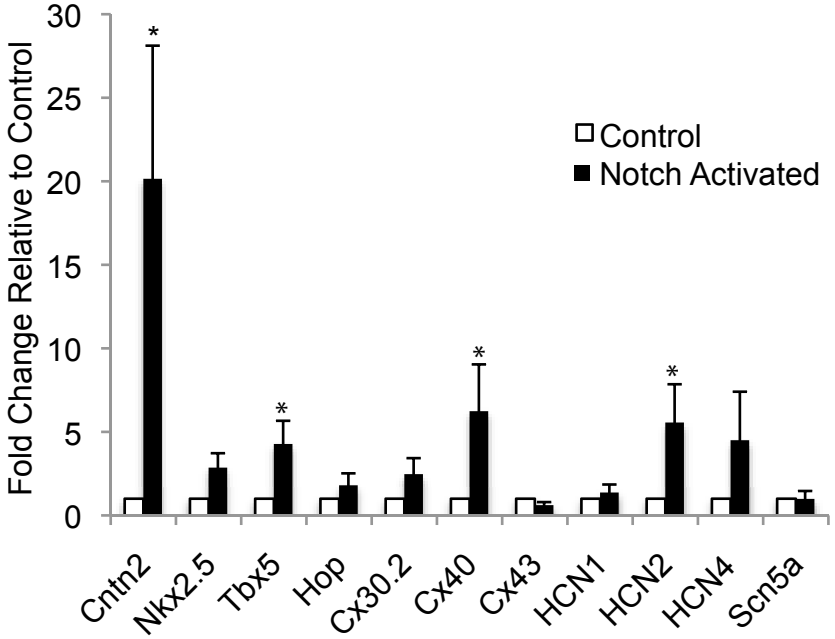


Supplemental Figure 6

Supplemental Figure 6. APD_{90} Distribution Reveals Heterogeneity *in vivo* **in Response to Notch Activation**

A total of 8 of 11 cells prolong their action potential and acquire a morphology characteristic of a Purkinje-like action potential in response to Notch activation. The APD_{90} for individual adult Notch activated *Mlc2v*$^{Cre/+}$; *Z/EG; NICD* and control *Mlc2v*$^{Cre/+}$; *Z/EG* cardiomyocytes is consistent with some Notch activated cells undergoing complete reprogramming (n=2/11), while others are incompletely reprogrammed. (n=6/11), or do not respond to Notch activation (n=3/11). APD_{90} prolongation more than 2 standard deviations above the average APD_{90} of ventricular cardiomyocytes was considered a response. The bar represents the average APD_{90} in each group (87.5 ms in *Mlc2v*$^{Cre/+}$; *Z/EG; NICD* vs. 41.0 ms in *Mlc2v*$^{Cre/+}$; *Z/EG).*

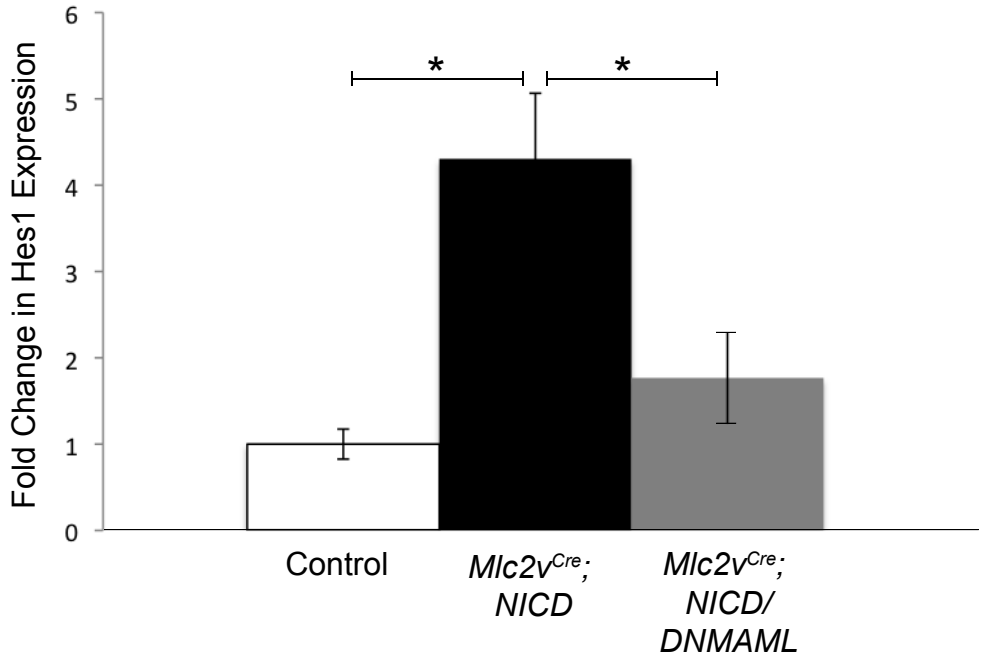




Supplemental Figure 7

Supplemental Figure 7. Notch Induces Similar Gene Expression Changes in Mid-Gestation Cardiomyocytes as in Perinatal Cardiomyocytes

(A) RT-qPCR analysis of induced gene expression changes to known Notch targets in 14.5 dpc crude ventricular cardiomyocyte preparations treated with activated Notch (black bars) versus control GFP-expressing adenovirus (white bars). (B) RT-qPCR analysis of conduction-enriched genes with Notch activation. A similar pattern of transcriptional changes is seen at 14.5 dpc when compared with Notch activation in newborn cardiomyocytes (Figure 3). n=4 replicates. Data are expressed as mean ± SEM. Group comparison was performed using a Student's unpaired 2-tailed *t*-test. *p<0.05



Supplemental Figure 8

Supplemental Figure 8. Inactivation of Canonical Notch Signaling by DNMAML Rescues Ectopic Up-regulation of Hes1

RT-qPCR analysis of *Hes1* gene expression changes within the mid-ventricular region of Notch activated hearts, with and without downstream inactivation of canonical Notch signaling via DNMAML (*Mlc2v$^{Cre/+}$; NICD* versus *Mlc2v$^{Cre/+}$; NICD/DNMAML*). Inactivation of canonical Notch signaling in Notch activated mice (*Mlc2v$^{Cre/+}$; NICD/DNMAML*) rescues up-regulation of *Hes1* by 58%. This is similar to the degree of rescue seen for *Hcn1* expression, which is decreased 64% in *Mlc2v$^{Cre/+}$; NICD/DNMAML* mice (Figure 5). Control mice are *NICD* or *NICD/DNMAML* littermates. n=9 control, n=8 *Mlc2v$^{Cre/+}$; NICD*, n=6 *Mlc2v$^{Cre/+}$; NICD/DNMAML.* Data are expressed as mean ± SEM. Group comparison was performed using a one-way ANOVA and Tukey-Kramer test for post-hoc analysis. *$p<0.05$