SUPPLEMENTAL MATERIAL

- Supplemental Methods

- Supplemental Figure 1. Related to Figure 1: Characterization of human adult and fetal CPCs
- Supplemental Figure 2. Related to Figure 1: DLL1-mediated activation of NOTCH signaling in adult CPCs
- Supplemental Figure 3. Related to Figure 1: Sequential Notch activation and inhibition in vitro
- Supplemental Figure 4. Related to Figure 1: J1-mediated activation of NOTCH signaling in adult CPCs
- Supplemental Figure 5. Related to Figure 2: Sequential Notch activation and inhibition in vivo
- Supplemental Figure 6. Related to Figure 3: Dependence on MIR-143/145
- Supplemental Figure 7. Related to Figure 5 and 6 Characterization of *CARMEN* isoform expression
- Supplemental Figure 8. Related to Figure 7: Transcriptome analysis

- Supplemental Tables 1. Related to Figure 1: Patients' characteristics
- Supplemental Tables 2. Related to Figure 7: RNA samples
- Supplemental Tables 3. Related to Figure 7: Predicted upstream regulators

- Supplemental Movie Legends

Supplemental Movie 1. Electrical stimulation of CPC-derived smooth muscle cells. Related to Figure 2. Cytosolic Ca^{2+} signals were recorded in differentiated adult CPCs, not exposed to DLL1 in the absence of DAPT. Cells were evaluated following electrical stimulation (10 s. at 0.5 Hz).

Supplemental Movie 2. Electrical stimulation of CPC-derived cardiomyocytes. Related to Figure 2. Cytosolic Ca^{2+} signals were recorded in differentiated adult CPCs, following exposure to DLL1 in the presence of DAPT. Cells were evaluated following electrical stimulation (10 s. at 0.5 Hz).

Supplemental Methods

Cloning

Freshly isolated CPCs were plated in 10-cm dishes under limiting dilution conditions. Cells giving rise to single colonies were visually identified. Each clonal population was individually harvested using cloning rings, and transferred into 48-well plates. Clones were grown as described above for primary cell cultures.

Coating

Dishes were incubated with antibodies anti-IgG-Fcγ (Goat anti-Mouse IgG Fcγ Fragment specific; Jackson Immuno research) over night on shaker at 4°C. Then, dishes were coated with IgG (Purified Human IgG: R&D, None), to obtain control dishes or with recombinant protein Recombinant Rat Delta-like1 Fc Chimera (R&D) for coated dishes with DLL1

Modulation of NOTCH signaling in CPCs

DLL1-mediated activation of NOTCH signaling in CPCs was essentially performed as described (36). Adult CPCs were plated in dishes coated with either purified human IgG (control) or with DLL1 for 24h. Cells were then switched to Differentiation medium supplemented with 5 μM of N-(N-(3,5-difluorophenacetyll)-L-alanyl)S-phenylglycine t-butyl ester (DAPT, Sigma-Aldrich), a NOTCH inhibitor diluted in Dimethylsulfoxide (DMSO, Sigma-Aldrich) or an equivalent amount of DMSO (No DAPT). The medium was changed every second day.

hN1 cells

hN1 cells, a reporter cell line, modified in order to express a Histone 2B-Citrine fluorescent (YFP) in response to Notch pathway activation were gently provided by Michael B. Elowitz {Sprinzak, 2010 #53} from Howard Hughes Medical Institute, California Institute of Technology, Pasadena, California. hN1 cells were expanded in a proliferation medium composed of MEM alpha medium Glutamax 1g/l glucose and ribonucleoside (Gibco), Fetal Calf Serum (10%), Penicillin Streptomycin (1%), Geneticin (600 μg/ml; Gibco), Zeocin (400mg/ml; Invitrogen), Blasticidin (10μg/ml; Invitrogen). Once they had a confluence of 80% approximately (every 2^{nd} or 3^{rd} days), cells were passed using Trypsin-EDTA 0.05% in PBS. Cells were centrifuged at 900G for 5min at a temperature of 18°C.

Immunofluorescence analysis

Cells in culture or OCT-embedded heart sections were fixed (2% paraformaldehyde in PBS) and permeabilized (0.3% Triton-X100 in PBS). Cells were incubated 1h in buffer containing 2.5% bovine serum albumin (Serva electrophoresis GmbH). The following antibodies were used in this study: human Nkx2.5 (mouse monolyclonal ; R&D systems ; 1:100), human lamin (mouse monoclonal, Novocastra; 1:300), GATA4 (rabbit polyclonal; Abcam; 1:200), MEF2C(rabbit polyclonal; Abcam; 1:200), phospho-Histone3 (rat polyclonal; pH3, Abcam; 1:200), the proliferation marker ki67 (rabbit polyclonal; Abcam; 1:200), sarcomeric α-actinin (mouse monoclonal ; Sigma ; 1:600), smooth muscle myosin heavy chain (smMHC; rabbit polyclonal, Biomedical Technologies Inc., 1:100), Alexa Fluor 488, Alexa Fluor 594 or Alexa Fluor 647 conjugated secondary antibodies specific to the appropriate species were used (Molecular Probes, 1:200). Nuclei were stained with DAPI (molecular probes). An Axiovision fluorescence microscope (Carl zeiss), a Nikon LSR Eclipse TE2000S inverted fluorescence microscope and a Zeiss LSM 710 Quasar Confocal Microscope were used in this study.

Flow cytometry

Cells were harvested and suspended in PBS containing 3% FCS, 2mM EDTA and incubated for 30' on ice with various antibodies. The following antibodies were used : FITC-conjugated anti-human CD31 (mouse monoclonal; BD Pharmingen), R-phycoerythrin (PE)-conjugated anti human CD45 (mouse monoclonal; BD Pharmingen), PE-Cy5-conjugated anti-human CD34 (mouse monoclonal; BD Pharmingen)**,** PE-conjugated anti-human CD117 (mouse monoclonal; BD Pharmingen), APC-conjugated anti-human CD73 (mouse monoclonal; eBioscience), APC-conjugated anti-human CD105 (mouse monoclonal; eBioscience), APC-conjugated anti-human CD90 (mouse monoclonal; BioLegend). The following antibodies were used as isotype controls: PE-conjugated mouse IgG1κ monoclonal immunoglobulin isotype control (BD Pharmingen), PE-Cy5-conjugated mouse IgG1κ monoclonal immunoglobulin isotype control (BD Pharmingen), FITC-conjugated mouse IgG1κ monoclonal immunoglobulin isotype control (BD Pharmingen), APC-conjugated mouse IgG1κ monoclonal immunoglobulin isotype control (eBioscience).

RNA sequencing and analysis

100nt paired-end reads from samples were mapped to the reference human genome using Tophat. Data normalization and differential expression analysis were performed in R (version 3.0.2). Raw counts were normalized using TMM (EdgeR R version 3.4.0) for genes with 1 count per million (cpm) in at least 1 sample.

Log transformation was applied on the normalized counts using Voom before using limma for differential expression (limma R 3.18.2). A moderated t-test was used for each comparison and adjusted p-values were computed by the Benjamini-Hochberg method, controlling for false discovery rate (FDR).

RNA Sequencing data

RNA Sequencing data are presented in the Supplemental Data Set 1.

Principle component analysis and hierarchical clustering

Principle component analysis and hierarchical clustering was performed on top modulated genes in CPCs differentiated into smooth muscle cells and in CPCs differentiated into cardiomyocytes following exposure to DLL1 and DAPT treatment.

GO analysis - Enrichment for biological themes

The genes lists were submitted Gene GO (https://portal.genego.com) for functional annotation using default parameters and databases for a single experiment analysis.

Predicted upstream regulators

Predicted upstream regulators for a set of seed genes were identified using Ingenuity Pathway Analysis (http://www.ingenuity.com).

Proliferation assay

CPC proliferation was assessed using a 5-ethynyl-2′-deoxyuridine (EdU) incorporation assay. Cells were incubated with 5 μM (final concentration) of EdU diluted in DMSO or with DMSO alone. One hour thereafter, the cells were rinsed, fixed and stained according to the manufacturer protocol (Invitrogen).

Confocal calcium imaging

Cells were grown on gelatin-coated glass coverslips and loaded with the fluorescent Ca^{2+} indicator fluo-3-AM (Biotium, 5 μM, 30 min., 10 min. deesterification). In order to elicit Ca^{2+} events in the cytosol, cells were electrically stimulated by field-stimulation. Changes in cytosolic Ca^{2+} levels are expressed as changes in fluorescence ($\Delta F/F_0$). Ca^{2+} images were acquired using a confocal microscope (FV1000, Olympus) with a 60x water-immersion objective. Fluo-3 was excited at 473 nm, and emitted fluorescence was collected at 490-540 nm. Movies were recorded at a rate of 2 frames per s. In order to elicit Ca^{2+} events in the cytosol, cells were

electrically stimulated by field-stimulation. Triggering pulses were 2.5 ms long with an amplitude 1.5x above threshold voltage (between 20 and 40 V) and a frequency of 0.5 Hz. Changes in intracellular Ca^{2+} during electrical pacing were recorded in frame images. Extracellular solution contained (in mM) 140 NaCl, 5.4 KCl, 1.8 $CaCl_2$, 1.1 $MgCl_2$, 5 HEPES and 10 Glucose, pH 7.4. Confocal images were analyzed in ImageJ and further processed using IgorPro. Changes in cytosolic Ca^{2+} levels are expressed as changes in fluorescence ($\Delta F/F_0$). In some experiments, calcium release was measured following caffeine addition (10 mM).

RNA extraction, RT-PCR and real-time PCR analysis

Total RNA was purified from each sample using Nucleospin RNA II Kit (Macherey Nagel) or miRNeasy kit (Qiagen) following the manufacturer's instructions. Total RNA was subjected to reverse transcription using oligodT (microsynth) and Moloney murine leukemia virus reverse transcriptase SuperScript III kit (Invitrogen) with random hexamer primers or miRCURY^{TM} LNA Universal RT microRNA PCR kit (Exiqon). Resulting cDNA was used as a template for RT-PCR or for relative quantitative PCR (qPCR) using specific probes and the ABI Prism 7500 Fast Real-Time PCR System (Applied Biosystems), or Exiqon SYBR Green PCR Kit for miRNA or Power Syber Green PCR master mix (Applied Biosystems) for LncRNA. Results from qPCR were normalized using the reference gene β2-microglobuline or U6 miRNA and the ΔΔCt method. Primers or probes used in the manuscript are described in the Tables below.

Primers used for RT-PCR

Gene	Forward Primer (5' to 3')	Reverse primer (5' to 3')
GATA4	5'GTGTCAACTGTGGGGCTATG3'	5'ATTCAGGTTCTTGGGCTTCC3'
MEF2C	5'CAGGACAAGGAATGGGAGGA3'	5'TGAGTAGAAGGCAGGGAGAGA3'
NKX2.5	5'CGACGCCGAAGTTCACGAAGT3'	5'CGCCGCTCCAGTTCATAG3'
α-MHC	5'TCTCTTCTCCTCCTACGCAAC3'	5'GCTTCTCTGTCCCCTTCCTG3'
β-MHC	5'CACCAACAACCCCTACGATT3'	5'ACTCATTGCCCACTTTCACC3'
TNNI	5'GAGAGATACGACATAGAGGCAAAA3'	5'CTCCTTCTTCACCTGCTTGA3'
Cx43	5'GGGGCAGGCGGGAAGCACCATCTC3'	5'TCTCTTATCCCCTCCCTCTCCACCCATCTACCC3'
GAPDH	5'ATCCCATCACCATCTTCCAG3'	5'TGAGTCCTTCCACGATACCA3'
α-satellite chromosome	5'AGCGATTTGAGGACAATTGC3'	5'CCACCTGAAAATGCCACAGC3'

Probes use for miRNA detection with 7500 Fast Real-Time PCR System

Probes	Sequence
U6	Not communicated
hsa-miR-143p	5'UGAGAUGAAGCACUGUAGCUC3'
hsa-miR-145p	5'GUCCAGUUUUCCCAGGAAUCCCU'

Probes from ABI

α-actin cardiac muscle	Hs00606316_m1
ATP2A2	Hs01566028_g1
b2-microglobuline	4333766T
CACNA1C	Hs00167681_m1
c-kit	Hs00174029_m1
CDKN1A;p21	Hs00355782_m1
cyclin D1	Hs00277039_m1
DLL1	Hs00194509_m1
DLL3	Hs01085096_m1
DLL4	Hs00184092_m1
elk-1	Hs00901847_m1
KLF-4	Hs00358836_m1
GATA4	Hs00171403_m1
Hand2	Hs00232769_m1
Hes1	Hs00172878_m1
Hey1	Hs01114113_m1
Hey2	Hs00232622_m1
Isl1	Hs01099685_m1
Jagged1	Hs00164982_m1
Jagged2	Hs00171432_m1
Mef2C	Hs00231149_m1
Mesp1	Hs00251489_m1
Mesp2	Hs02742533_m1
v-Myc	Hs00153408_m1
MYH11; sm-MHC	Hs00224610_m1
MYH6; a-MHC	Hs01101425_m1
MYH7; b-MHC	Hs01110632_m1

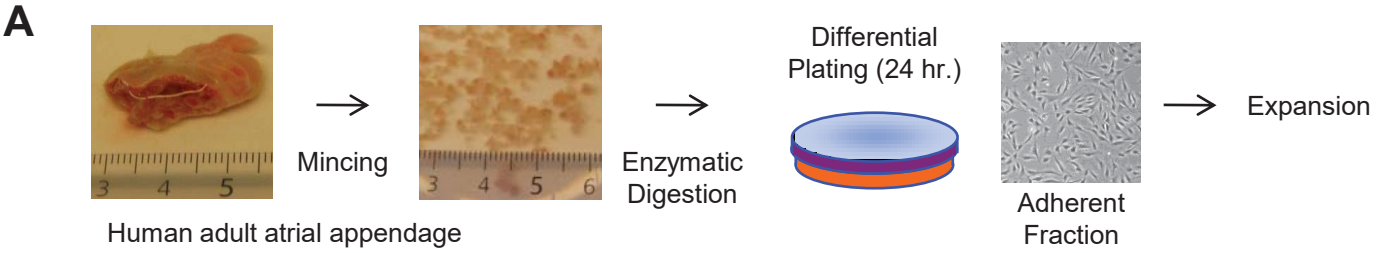
Myocardin	Hs00538071_m1
Nkx2.5	Hs00231763_m1
Notch1	Hs00413187_m1
Notch2	Hs01050719_m1
Notch3	Hs01128541_m1
Notch4	Hs00965889_m1
PCNA	Hs00696862_m1
RYR2 (cardiac)	Hs00892842_m1
SRF	Hs00182371_m1
Tbx20	Hs00396596_m1
Tbx5	Hs01052563_m1
Tnni3; TroponinI	Hs00165957_m1

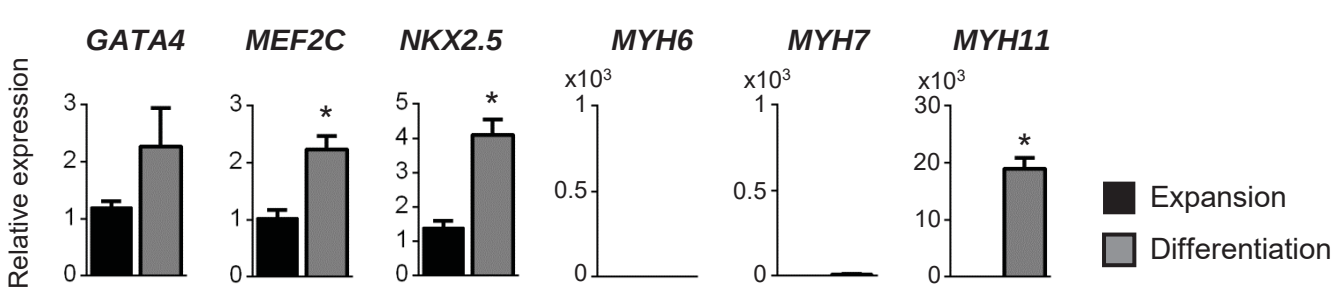
Probes used for LncRNA detection with 7500 Fast Real-Time PCR System

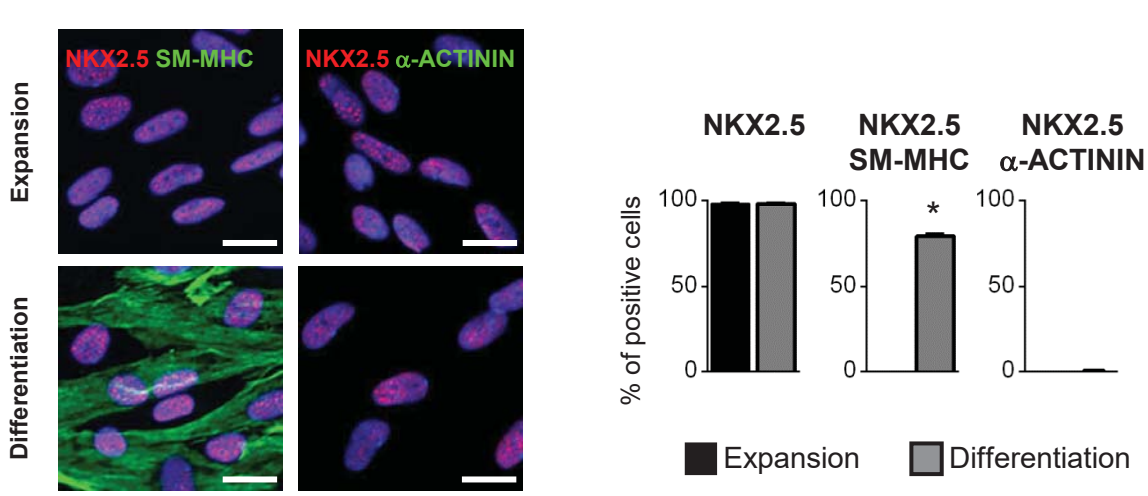
Gene	Forward Primer (5' to 3')	Reverse primer (5' to 3')
CARMEN1	5'CCAACCACTCCCCAAACA3'	5'TTACAGCCGTTGCTCTCCTT3'
CARMEN2	5'GATGGAAAAGATGGCAGAGG3'	5'TAGGTGTTGGCTGAGTGCAG3'
CARMEN3	5'AAGGAGAGCAACGGCTGTAA3'	5'GGAGGCTGCTTCTCCAGAGT3'

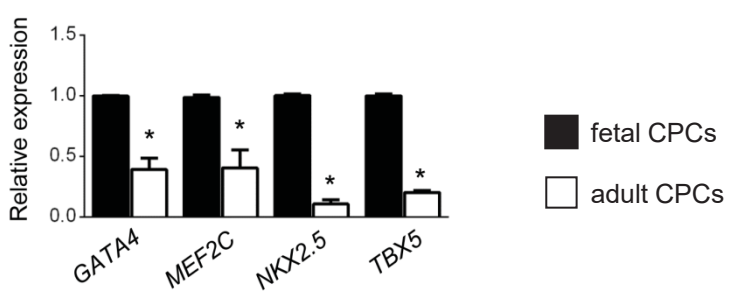
Cell transfection

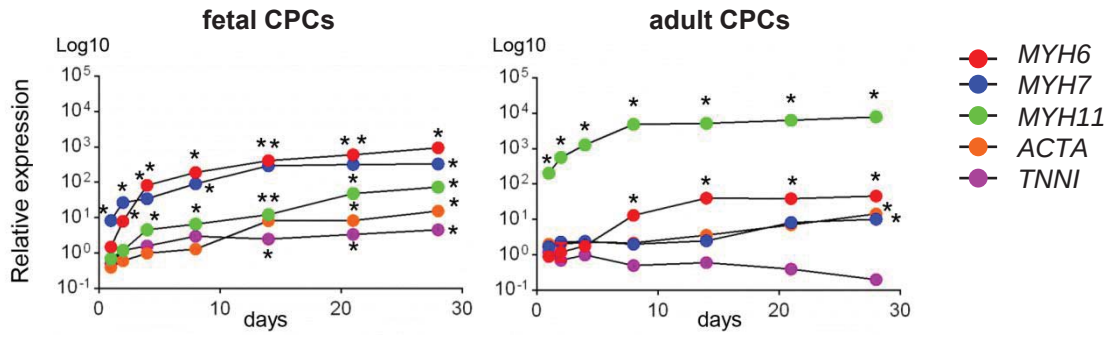
Transfection of adult CPCs with scrambled, anti-*MIR-143* or anti-*MIR-145* LNA inhibitors (Exiqon), and with scrambled, MIR-143 or MIR-145 mimics (Thermoscientific Dharmacon), was performed using 50 nM of the respective oligonucleotide or 25 nM of both oligonucleotides together with Fugene (Roche) according to manufacturer's instructions. The transfection of scrambled GapmeRs or GapmeRs targeting the three *CARMEN* isoforms (Exiqon) was performed using 100nM of GapmeR (Exiqon). Cells were processed for RNA extraction and immunostaining 48 h, respectively 10 days after transfection.





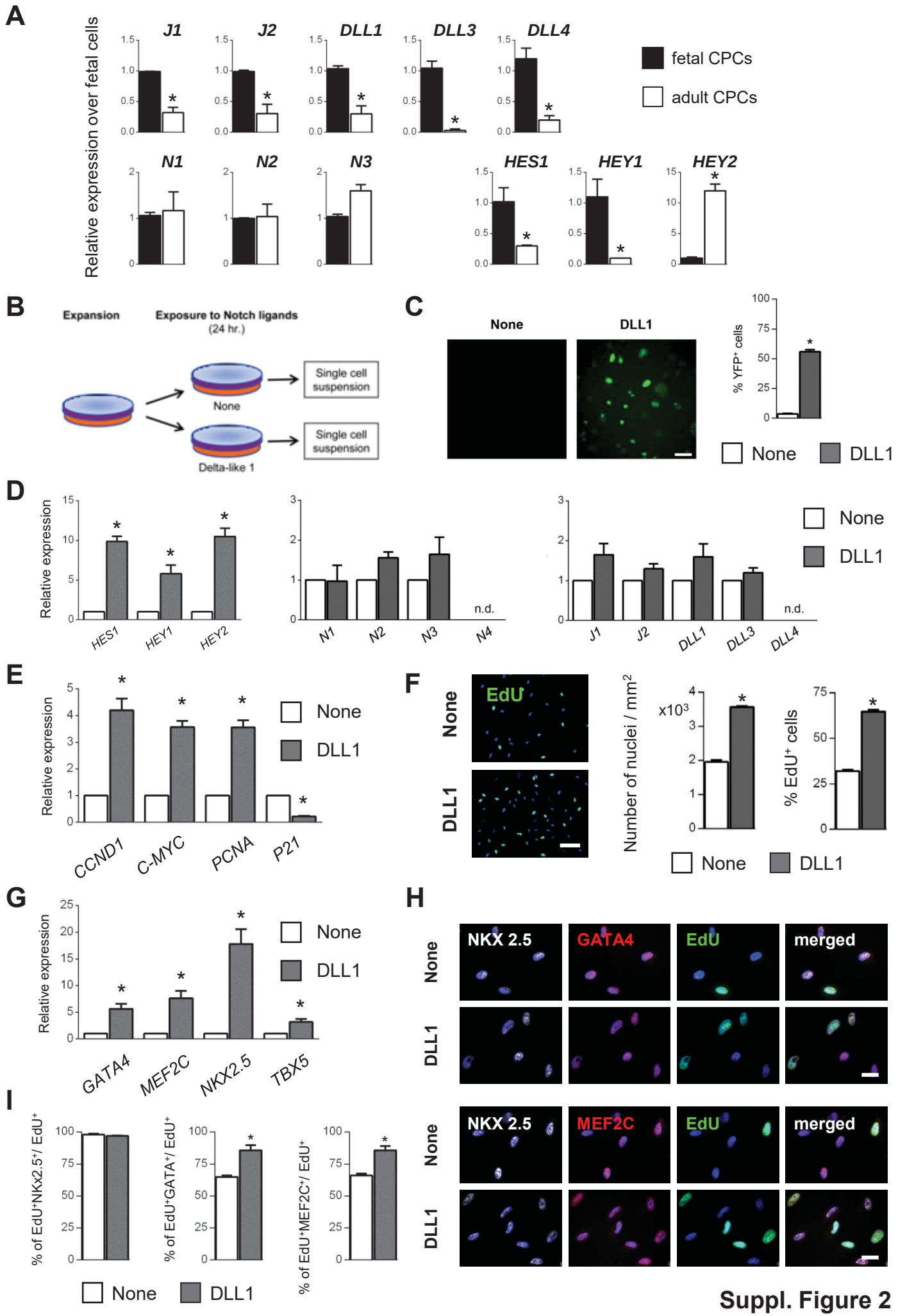






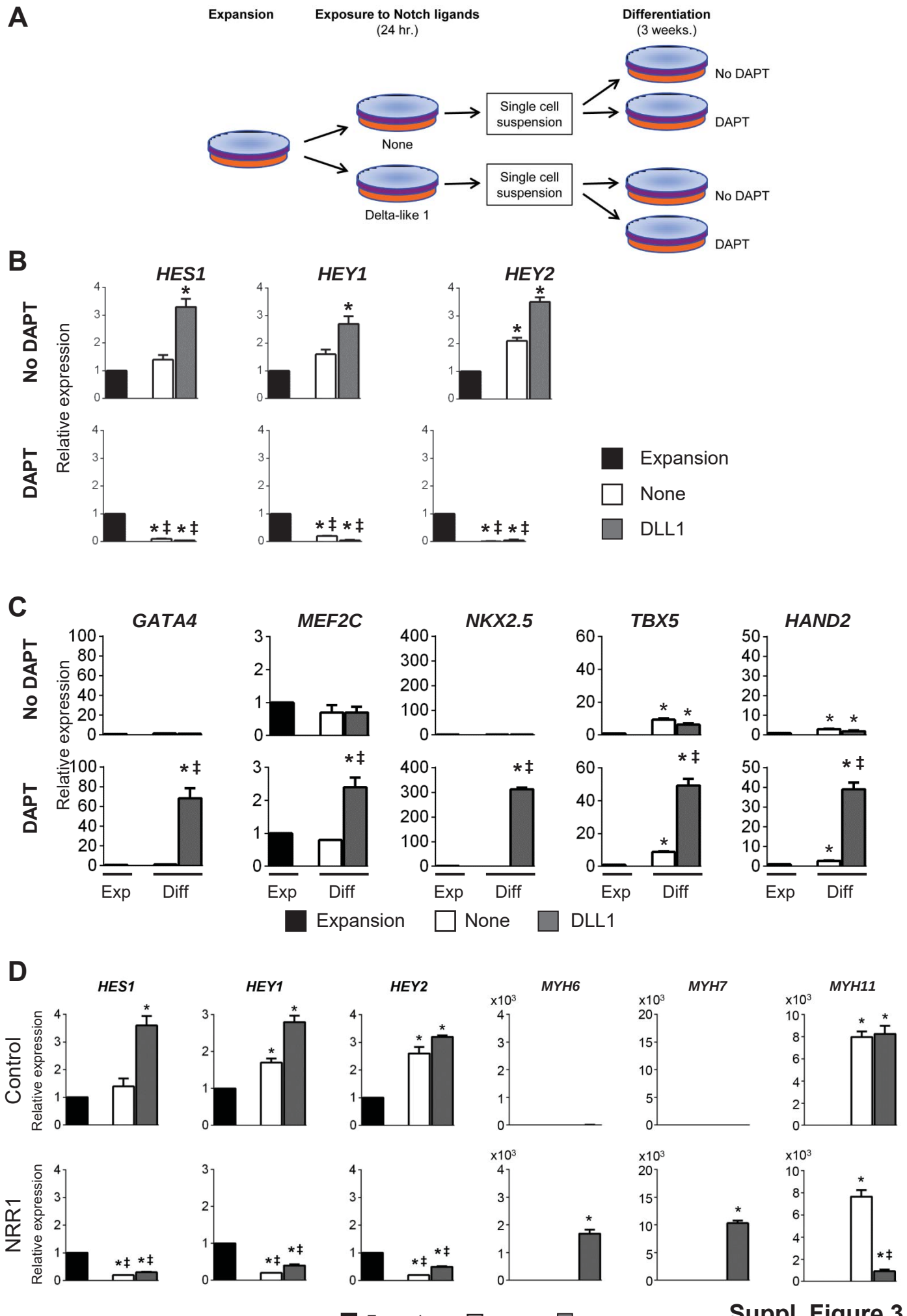
Suppl. Figure 1

Supplemental Figure 1. Characterization and differentiation of adult CPCs. (A) Schematic of adult CPC isolation. (B) Expression of early (*GATA4*, *MEF2C, NKX2.5*) and late cardiac markers *(MYH6*, *MYH7*, *MYH11*) in differentiating adult CPCs. Expansion (black); Differentiation (gray). Data represent means ± SEM; * $p<0.05$ (n=6). (C) Analysis of NKX2.5, sm-MHC and α-ACTININ expression by immunostaining. Nuclei were stained with DAPI (blue). Quantification of the number of $NKX2.5^{POS}$; $NKX2.5^{POS}$/sm-MHC^{POS}; $NKX2.5^{POS}$/α-$ACTININ^{POS}$ CPCs under proliferating conditions and following induction of differentiation. Data represent means ± SEM; * $p<0.05$. Scale bar: 20 μm (min. 2000 cells in 30 different fields at a magnification of 40X per quantification). (D) Expression of early cardiac markers (*GATA4*, *MEF2C, NKX2.5, TBX5*) in fetal and adult CPCs under expansion conditions. Data represent means ± SEM; * $p<0.05$ as compared to fetal cells (n=3). (E) Time course of sarcomeric protein expression *(MYH6*, *MYH7, MYH11*, *ACTA*, *TNNI*) during CPC differentiation. Data represent means ± SEM. * $p<0.05$ as compared to proliferating CPCs (example of one experiment performed in triplicates).



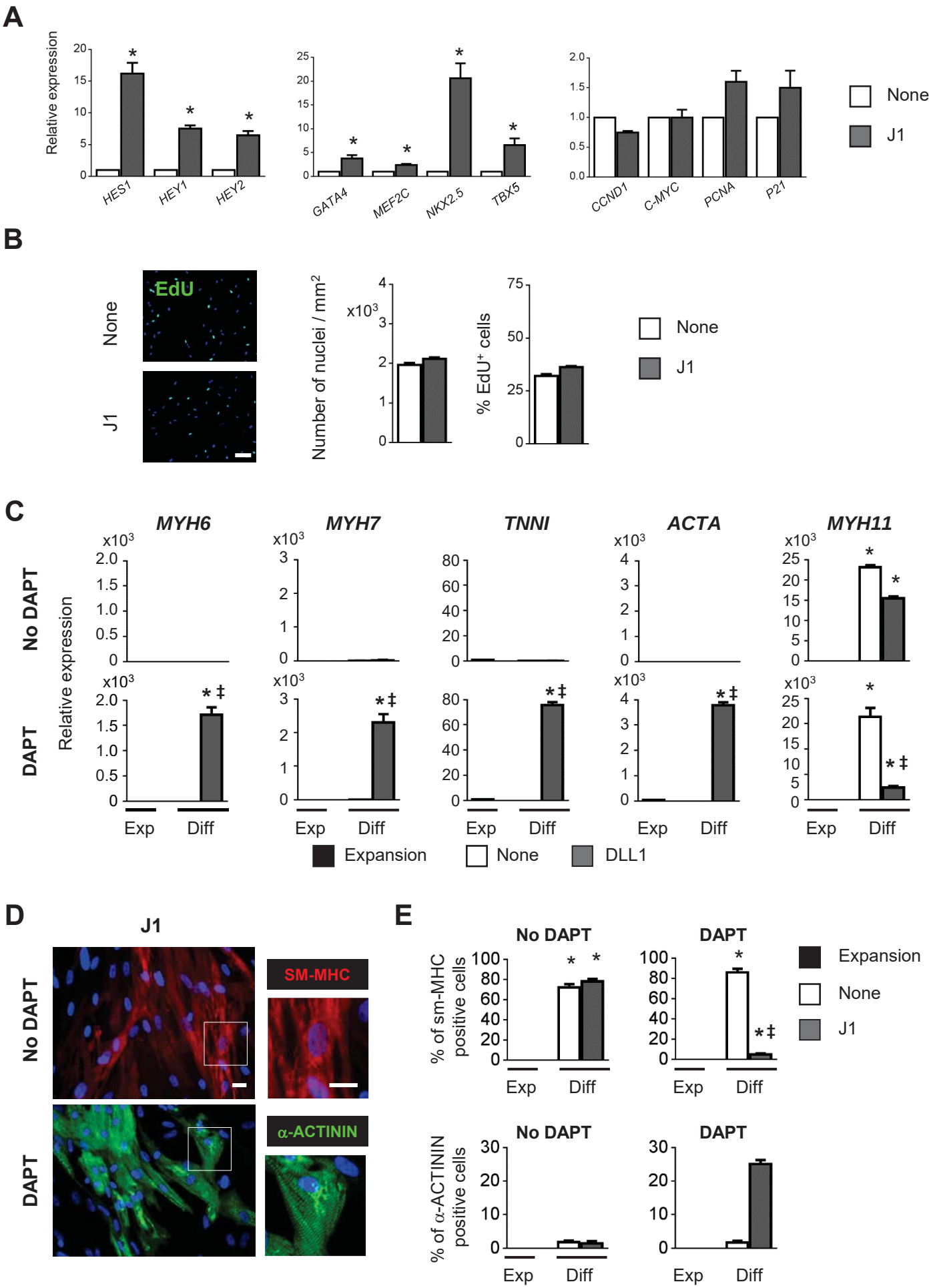
Suppl. Figure 2

Supplemental Figure 2. Notch stimulation induced proliferation of adult CPCs. (A) Expression of components of the Notch pathway (*Jagged*(*J)1*, *J2*, *DELTA-LIKE(D)LL1*, *DLL3*, *DLL4*, *NOTCH(N)1*, *N2*, *N3*, *N4*) in fetal and adult CPCs under expansion conditions. Data represent means ± SEM; * p<0.05 compared to fetal cells (n=3). (B) Schematic of DLL1-mediated Notch stimulation in adult CPCs. (C) Detection of YFP reporter expression in unstimulated (None) or DLL1-stimulated hN1 cells. Quantification of the number of YFP^{POS} hN1 following exposure to DLL1 (gray) (n=2). (D) Expression of components of the Notch pathway (*Jagged*(*J)1*, *J2*, *DELTA-LIKE(D)LL1*, *DLL3*, *DLL4*, *NOTCH(N)1*, *N2*, *N3*, *N4*) in adult CPCs following exposure to DLL1 (gray). Expression of Notch target genes (*HES1; HEY1; HEY2*) in adult CPCs exposed to DLL1 (gray). Data represent means ± SEM; * p<0.05 as compared to not stimulated cells (None; white) (n=5). (E) Expression of regulators of the cell cycle (*CCND1; C-MYC; PCNA; P21)* in adult CPCs exposed to DLL1 (gray). Data represent means ± SEM; * p<0.05 as compared to not stimulated cells (None; white) (n=5). (F). Proliferation in unstimulated (None) or DLL1-stimulated (DLL1) CPCs as measured by EdU incorporation and immunodetection (green). Quantification of the total number of CPCs (DAPI-positive cells) and of the number of proliferating CPCs (EdU^{POS}) following exposure to DLL1. Data represent means ± SEM; * p<0.05 as compared to not stimulated cells (None; white) (min. 3000 cells in 20 different fields at a magnification of 20X per quantification). Scale bar: 100 µm. (G) Expression of cardiac transcription factors (*GATA4; MEF2C; NKX2.5; TBX5)* in adult CPCs exposed to DLL1 (gray). Data represent means ± SEM; * p<0.05 as compared to not stimulated cells (None; white) (n=5). (H) Analysis of NKX2.5 and GATA4 expression, and EdU incorporation by immunostaining in CPCs exposed or not to DLL1. Nuclei were stained with DAPI (blue). Scale bars: 50 µm. (I) Quantification of the number of $NKX2.5^{POS}$ and $GATA4^{POS}$ proliferating (EdU^{POS}) adult CPCs following exposure to DLL1. Data represent means ± SEM; * p<0.05 as compared to not stimulated cells (None; white) (min. 2000 cells in 30 different fields at a magnification of 40X per quantification).



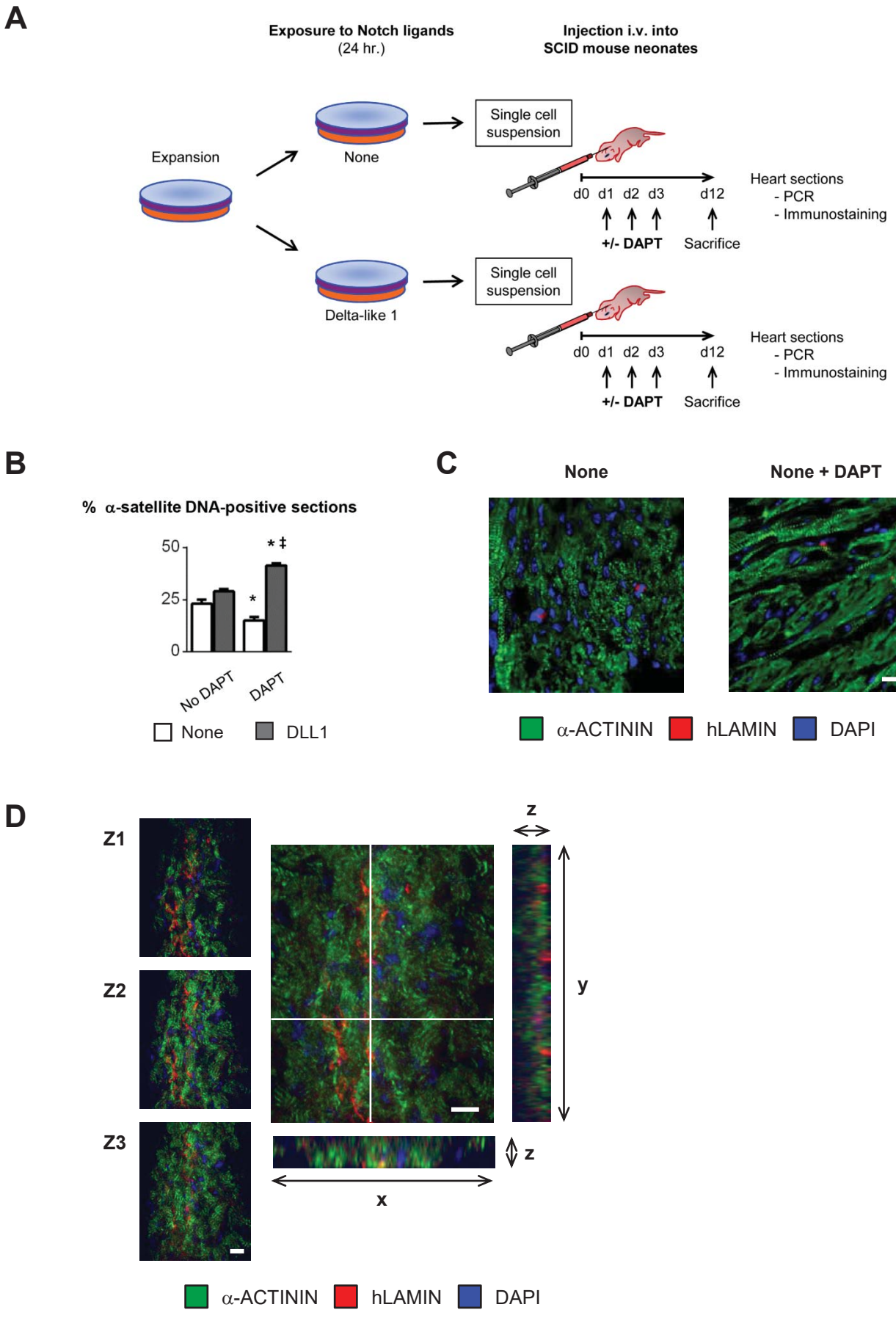
Suppl. Figure 3

Supplemental Figure 3. Specification and differentiation of Notch-stimulated adult CPCs following exposure to immobilized DLL1 ligand. (A) Schematic of CPC differentiation following exposure to DLL1 in the absence or presence of DAPT. (B) Expression of Notch target genes (*HES1; HEY1; HEY2*) in proliferating CPCs (Expansion; black) and in adult CPCs exposed to DLL1 (gray), and differentiated in the absence (upper panels) or presence of DAPT (lower panels). Data represent means ± SEM; * $p<0.05$ as compared to proliferating CPCs; ‡ $p<0.05$ as compared to differentiating unstimulated (None; white) CPCs in absence of DAPT (n=5). (C) Expression of cardiac transcription factors (*GATA4; MEF2C; NKX2.5; TBX5; HAND2*) in proliferating CPCs (Expansion; black) and in adult CPCs exposed to DLL1 (gray), and differentiated in the absence (upper panels) or presence of DAPT (lower panels). Data represent means ± SEM; * $p<0.05$ as compared to proliferating CPCs; ‡ $p<0.05$ as compared to differentiating unstimulated (None; white) CPCs in absence of DAPT (n=5). (D) Expression of Notch target genes (*HES1; HEY1; HEY2*) or sarcomeric proteins (*MYH6; MYH7; MYH11*) in proliferating CPCs (Expansion; black) and in adult CPCs exposed to DLL1 (gray), and differentiated in the presence of an isotype control antibody (Control), or antibodies directed against N1 (NRR1). Data represent means ± SEM; * $p<0.05$ as compared to proliferating CPCs; ‡ $p<0.05$ as compared to differentiating unstimulated (None; white) CPCs in absence of DAPT (n=3).



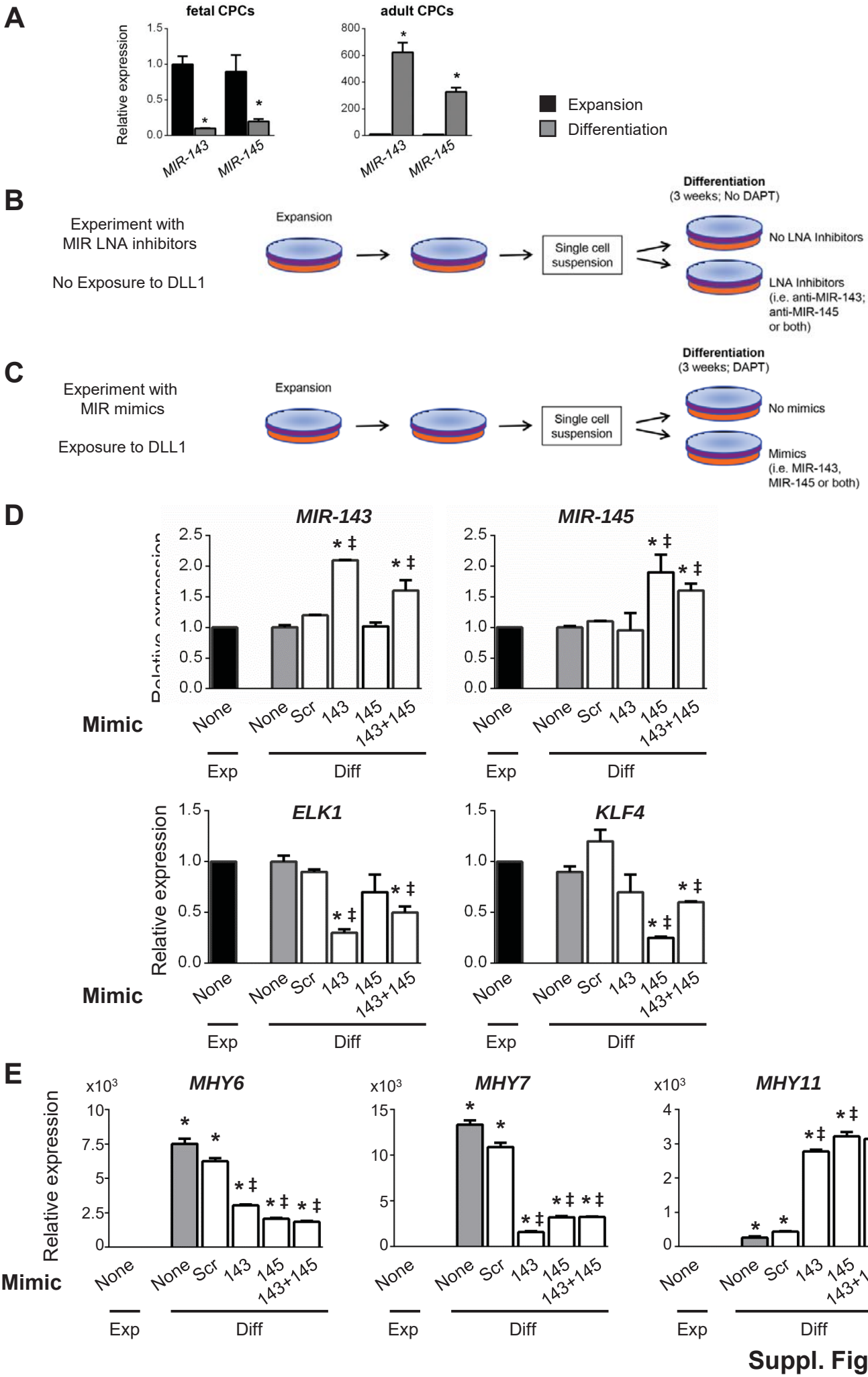
Suppl. Figure 4

Supplemental Figure 4. Notch activation by J1 followed by inhibition of Notch signaling promotes differentiation of adult CPCs into cardiomyocytes. (A) Expression of Notch target genes (*HES1; HEY1; HEY2*), cardiac transcription factors (*GATA4; MEF2C; NKX2.5; TBX5),* or regulators of the cell cycle (*CCND1; C-MYC; PCNA; P21)* in adult CPCs exposed to J1 (gray). Data represent means ± SEM; * $p<0.05$ as compared to unstimulated cells (None; white) (n=6). (B) Proliferation of unstimulated (None) or J1-stimulated (J1) CPCs as measured by EdU incorporation (green). Quantification of the number of proliferating CPCs (EdU^{POS}) following exposure to J1. Data represent means ± SEM; * $p<0.05$ as compared to unstimulated cells (None; white) (>5000 cells in 40 different fields at a magnification of 20X per quantification). Scale bar: 100 μm.(C) Expression of *MYH6*, *MYH7, TNNI, ACTA*, and *MYH11* in proliferating CPCs (Expansion; black), and in adult CPCs exposed to J1 (gray) and differentiated in the absence (upper panels) or presence of DAPT (lower panels). Data represent means ± SEM; * $p<0.05$ as compared to proliferating CPCs; ‡ $p<0.05$ as compared to differentiating unstimulated (None; white) CPCs in absence of DAPT (n=5). (D) Analysis of SM-MHC and α-ACTININ expression by immunostaining in CPCs exposed or not to J1 and differentiated in the absence or presence of DAPT. Nuclei were stained with DAPI (blue). Scale bars: 20 μm (E) Quantification of $SM\text{-}MHC^{POS}$ and $\alpha\text{-}ACTININ^{POS}$ in adult CPCs exposed to J1 (gray) and differentiated in the absence or presence of DAPT. Data represent means ± SEM; * $p<0.05$ as compared to proliferating CPCs; ‡ $p<0.05$ as compared to differentiating unstimulated (None; white) CPCs in absence of DAPT (>2000 cells in 30 different fields at a magnification of 40X per quantification).



Suppl. Figure 5

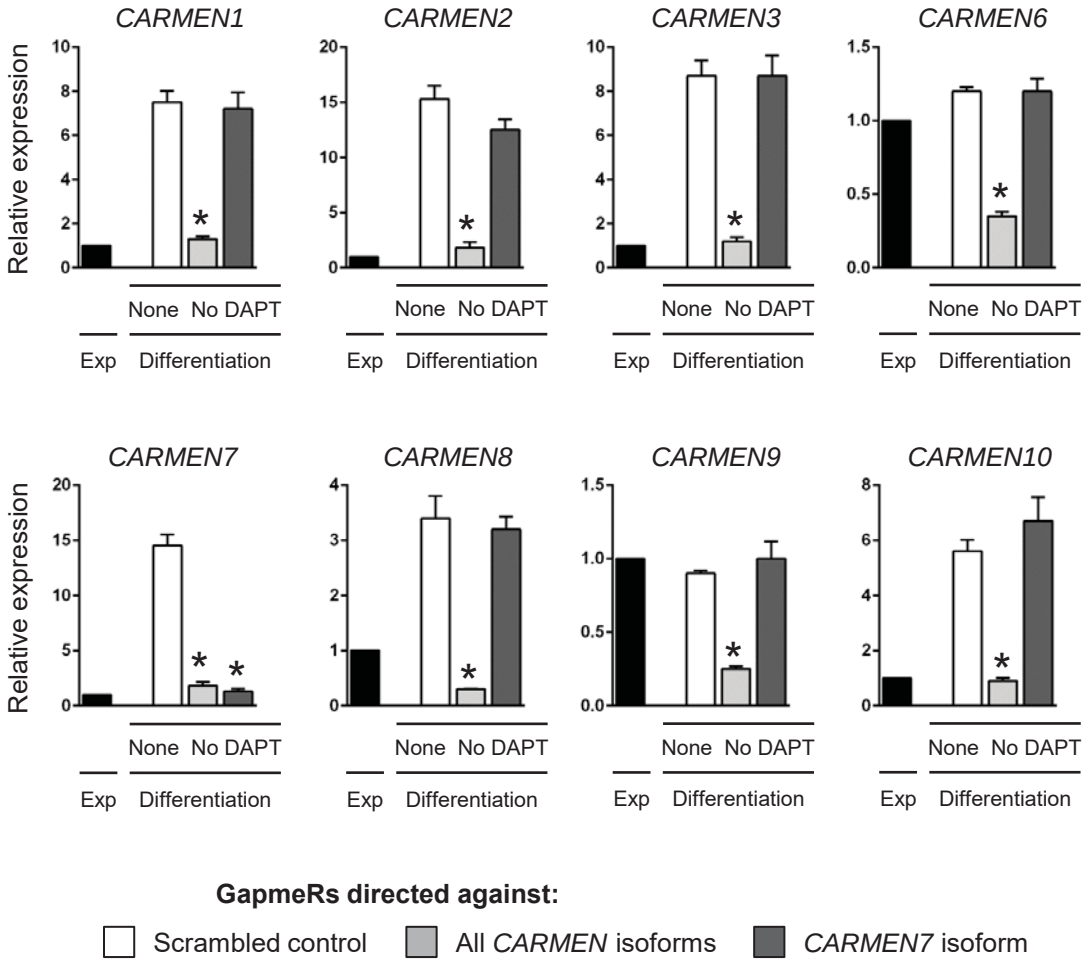
Supplemental Figure 5. Sequential activation and inhibition of the Notch pathway in adult CPCs promotes differentiation into cardiomyocytes in vivo. (A) Schematic of CPCs injection into SCID mice. Unstimulated (None) or DLL1-stimulated CPCs were injected into neonatal SCID mice. Mice were administrated with DAPT or vehicle alone. (B) Satellite human chromosome was detected by PCR in heart sections. The percentage of positive sections was determined in the indicated groups. Data represent means ± SEM; * $p<0.05$; as compared to mice that received unstimulated (None) CPCs in absence of DAPT. ‡ $p<0.05$; as compared to mice that received DLL1-stimulated CPCs in the absence of DAPT. One hundred sections analyzed per animal and 5 animals per group. (C) Analysis of human (h)LAMIN (red) and α-ACTININ expression (green) in SCID mouse hearts by immunostaining in control animals that received human CPCs not exposed to DLL1, and were administrated with DAPT or vehicle alone. Nuclei were stained with DAPI (blue). Scale bars: 20 μm. (D) Z stack analysis of confocal images of heart sections from mice that received DLL1-stimulated CPCs and were administrated with DAPT. Scale bars: 50μm.



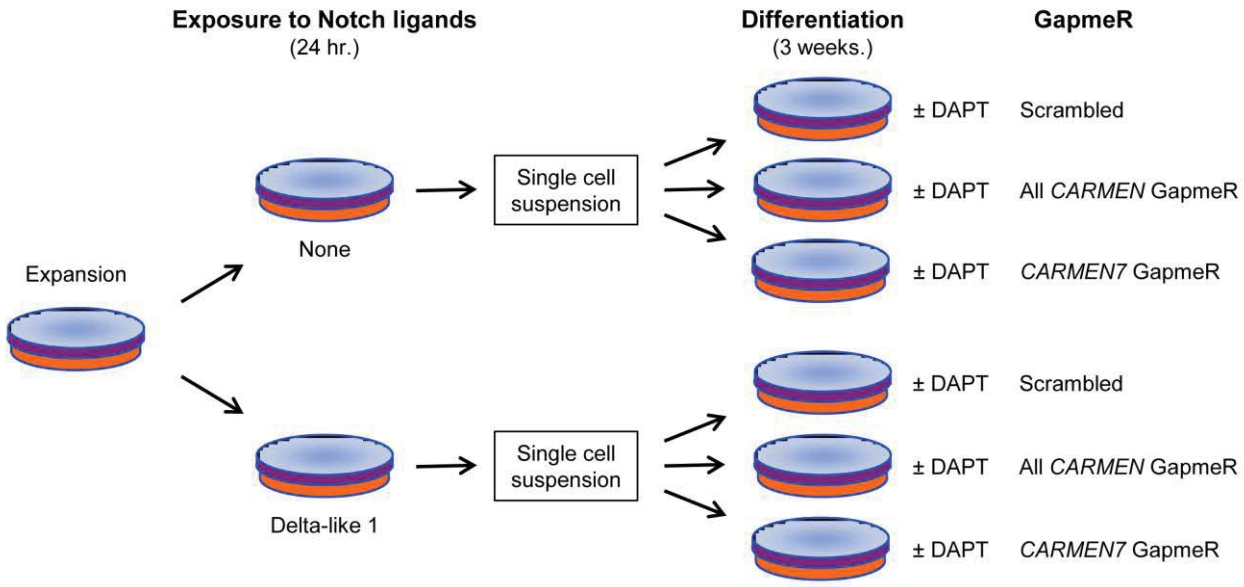
Suppl. Figure 6

Supplemental Figure 6. Differentiation of adult CPCs into cardiomyocytes or smooth muscle cells depends on MIR-143/145 expression. (A) Expression of *MIR-143* and *MIR-145* in proliferating (Expansion; black) and in differentiated fetal and adult CPCs (Differentiation, gray). Data represent means ± SEM; * $p<0.05$ as compared to proliferating CPCs (n=4). (B) Schematic of CPC transfection with MIR-143 or MIR-145 LNA inhibitors in a non–cardiogenic protocol (No exposure to DLL1, No DAPT). (C) Schematic of CPC transfection with MIR-143 or MIR-145 mimics in a cardiogenic protocol (Exposure to DLL1, DAPT). (D) Expression of *MIR-143*; *-145* and miRNA targets (*ELK1; KLF4*) in proliferating CPCs (Expansion; black) and in differentiated adult CPCs (exposed to DLL1, in the presence of DAPT). Cells were either transfected (white) or not (None; gray) with the indicated miRNA mimic. Data represent means ± SEM; * $p<0.05$ as compared to proliferating CPCs; ‡ $p<0.05$ as compared to differentiating CPCs transfected with scrambled mimic (n=3). (E) Expression of sarcomeric proteins (*MYH6; MYH7; MYH11*) in proliferating CPCs (Expansion; black) and in differentiated adult CPCs exposed to DLL1 in the presence of DAPT. Cells were transfected (white) or not (None; gray) with the indicated miRNA mimic. Data represent means ± SEM; * $p<0.05$ as compared to proliferating CPCs; ‡ $p<0.05$ as compared to differentiating CPCs transfected with scrambled mimic (n=3).

A

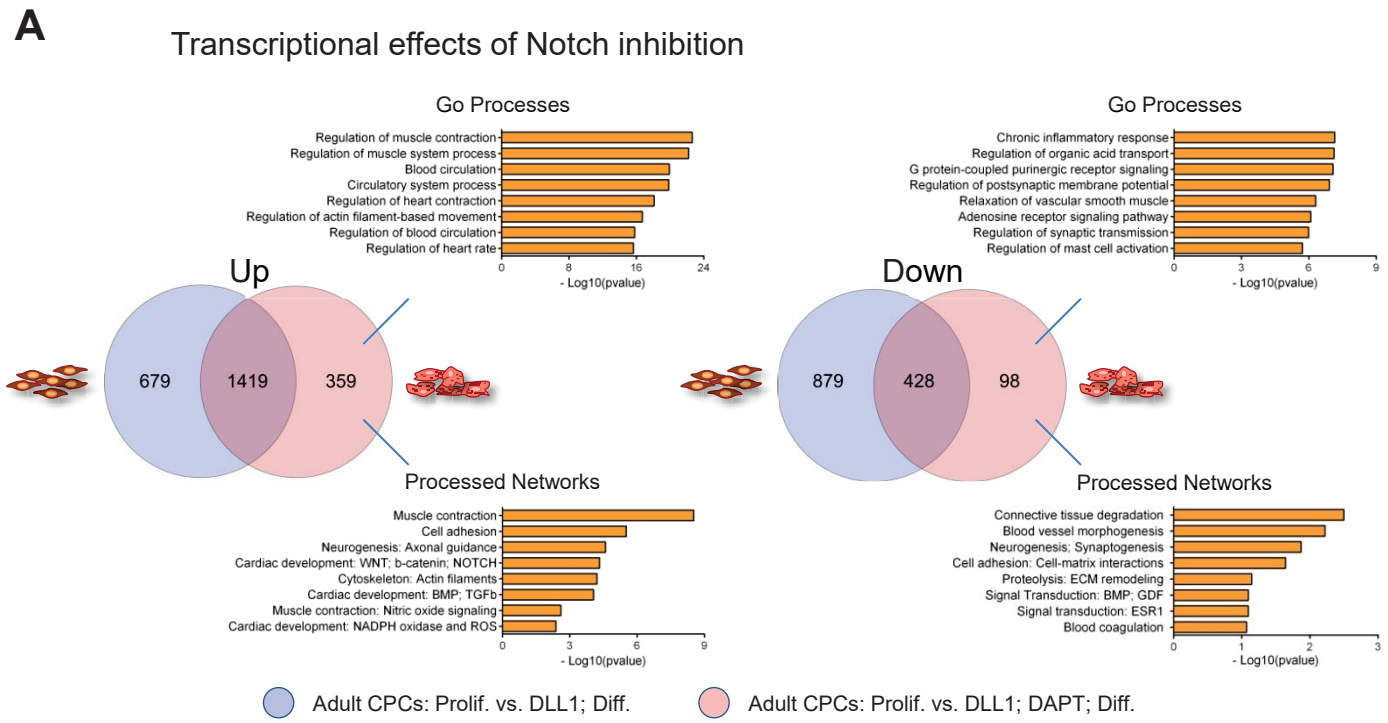


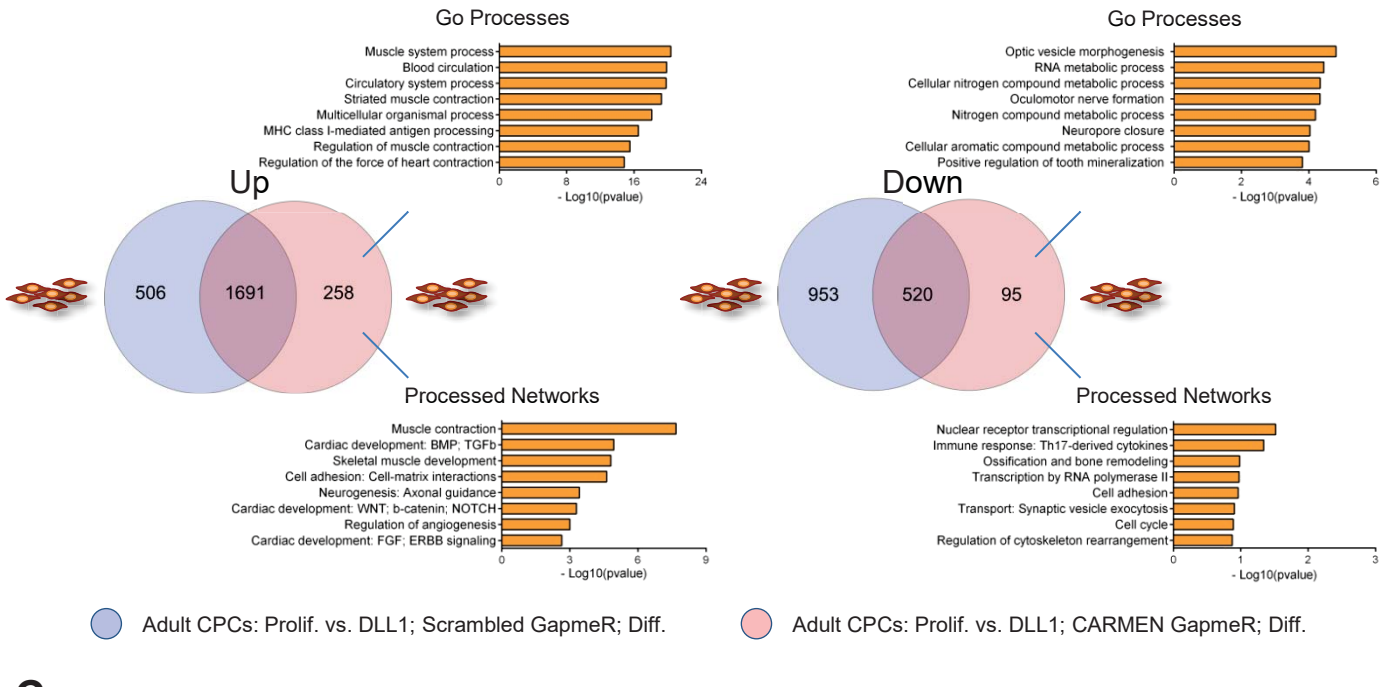
B

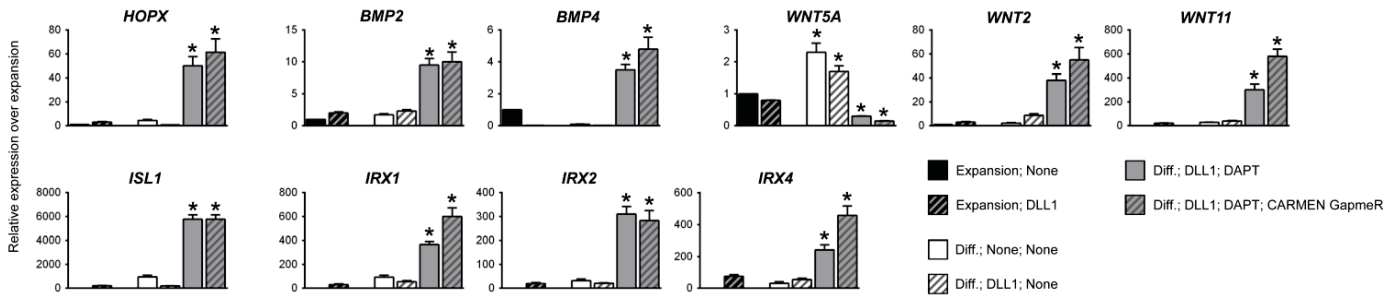


Suppl. Figure 7

Supplemental Figure 7. (A) Expression of *CARMEN1; CARMEN2; CARMEN3, CARMEN6; CARMEN7; CARMEN8 , CARMEN9* and *CARMEN10* in proliferating (Exp) and differentiating adult CPCs without prior DLL1 stimulation (None) and cultured in the absence of DAPT. Cells are transfected with either scrambled GapmeRs, GapmeRs directed against all *CARMEN* isoforms or GapmeRs directed specifically against *CARMEN7* before inducing differentiation. Data represent means ± SEM; * $p<0.05$ as compared to proliferating CPCs (n=3). (B) Schematic of CPC transfection with either scrambled GapmeRs, GapmeRs directed against all *CARMEN* isoforms or GapmeRs directed specifically against *CARMEN7* during cardiogenic differentiation.







Suppl. Figure 8

Supplemental Figure 8. (A) Transcriptional effects of Notch inhibition. Venn diagram of up- and downregulated genes unique to adult CPCs differentiating into cardiomyocytes following exposure to DLL1 and DAPT-mediated Notch inhibition (red). Adult CPCs exposed to DLL1 but differentiated in the absence of DAPT, thereby producing smooth muscle cells, are used as control (blue). Gene ontology terms and biological processes annotated by Gene Go are indicated in each case. (B). Transcriptional effects of *CARMEN* inhibition. Venn diagram of up- and downregulated genes unique to adult CPCs differentiating in smooth muscle cells further to exposure to DLL1 and transfection with GapmeRs directed against *CARMEN* in the absence of DAPT (red). Adult CPCs exposed to DLL1, transfected with scrambled GapmeRs and cultured in the absence of DAPT, thereby producing smooth muscle cells, are used as control (blue). Gene ontology terms and biological processes annotated by Gene Go are indicated in each case. (C) Expression of *HOPX, BMP2, BMP4, WNT5A, WNT2, WNT11, ISL1, IRX1, IRX2* and *IRX4* in proliferating adult CPCs (Expansion; None, black), in proliferating adult CPCs exposed to DLL1 (Expansion; DLL1, striped black), in differentiating adult CPCs (Diff.; None; None, white), in differentiating adult CPCs following exposure to DLL1 (Diff.; DLL1; None, striped white), in differentiating adult CPCs following exposure to DLL1 and in the presence of DAPT (Diff.; DLL1; DAPT, gray) and in differentiating adult CPCs, transfected with GapmeR targeting *CARMEN*, following exposure to DLL1 in the presence of DAPT (Diff.; DLL1; DAPT; CARMEN GapmeR, striped gray). Data represent means ± SEM; * $p<0.05$ as compared to proliferating CPCs (black bars) (n=3).