Stem Cell Reports

Supplemental Information

Efficient Generation of Cardiac Purkinje Cells from ESCs by Activating cAMP Signaling

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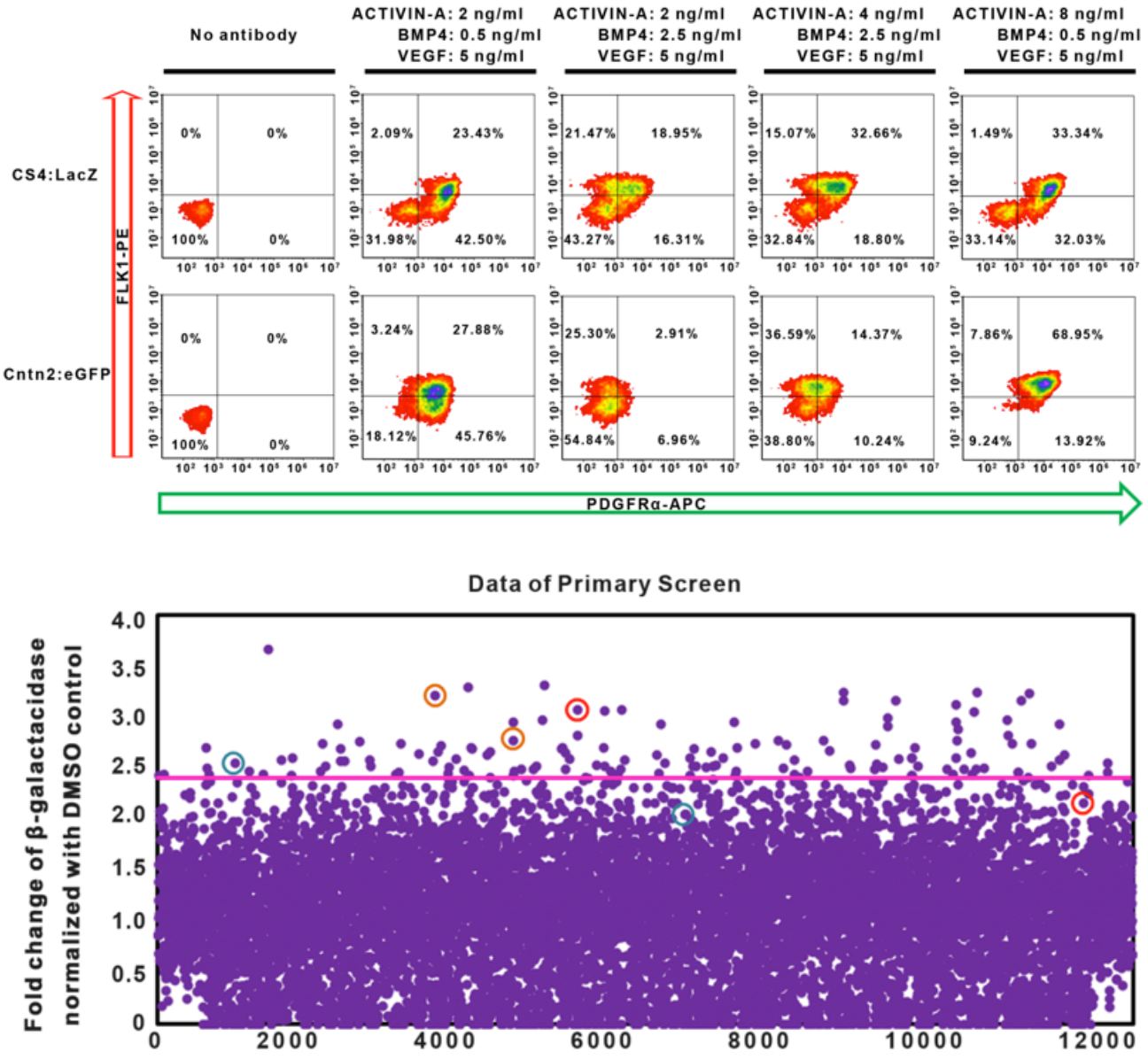
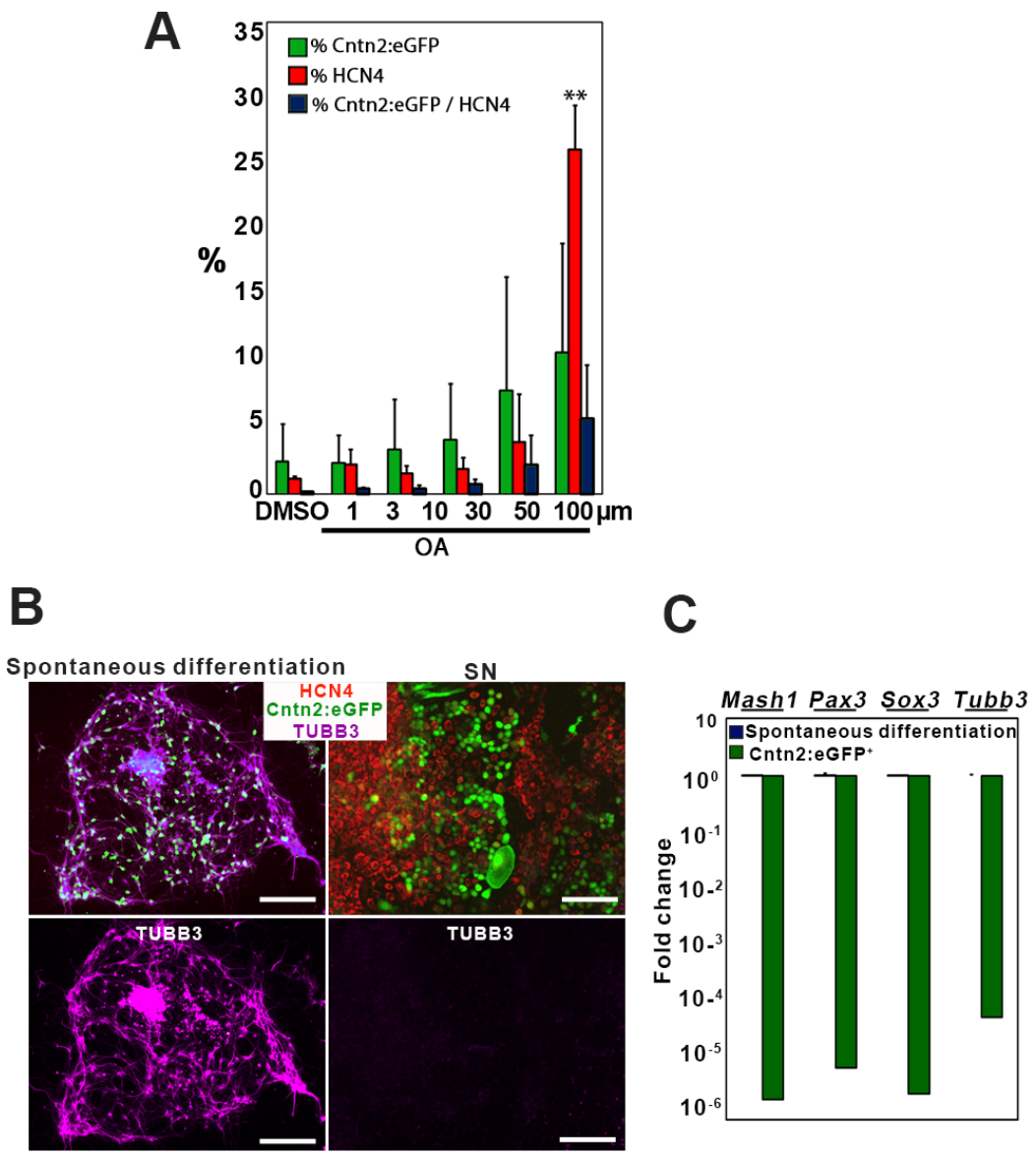


Supplemental Figure S1. Optimization of cytokine induction for reporter ESC lines and primary screen results. Top: Flow plots of FLK-1 and PDGFR-α expression. CS4:LacZ and Cntn2:eGFP ESC cell lines were treated with indicated concentrations of BMP4, ACTIVIN-A and VEGF and the FLK1 and PDGFR-α expression in both lines evaluated by flow cytometry analysis. CS4:LacZ cells and Cntn2:eGFP cells are shown in the upper and lower panels, respectively. PDGFR-α^+ and FLK1$^+$ expression is shown in the X-axis and Y-axis, respectively.

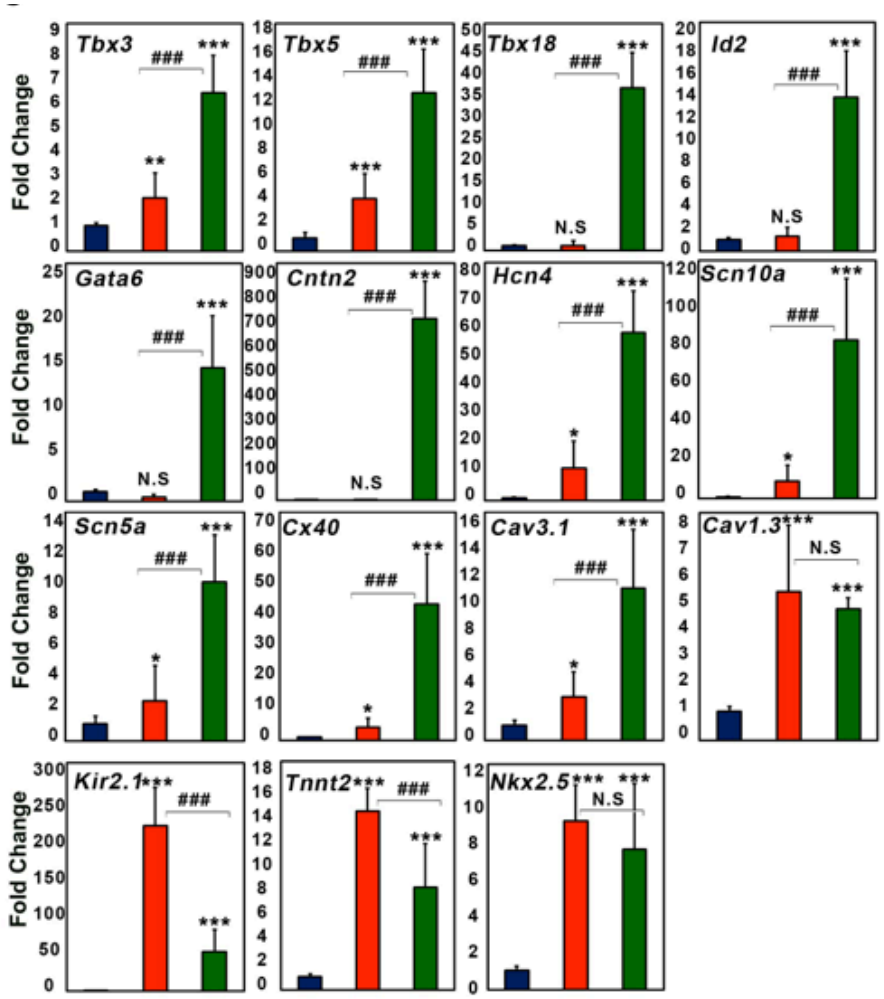
Bottom: Each dot represents one compound at one concentration. Approximately 5,000 compounds were examined at two different concentrations (10 and 1 μM). Y-axis shows fold change of β-galactosidase activity normalized to DMSO control. Primary hit compounds that had 2.5 fold or more increasing levels of β-galactosidase activity were selected (above pink line). Three primary hit compounds were highlighted by different colors of circles. Red circles (SN treatment), orange circles (OA treatment) and blue circles (CH treatment) are indicated.



Supplemental Figure S2. Oleic acid enhances cardiac conduction system marker HCN4 expression and Cntn2:eGFP$^+$ cells are not neural cells.

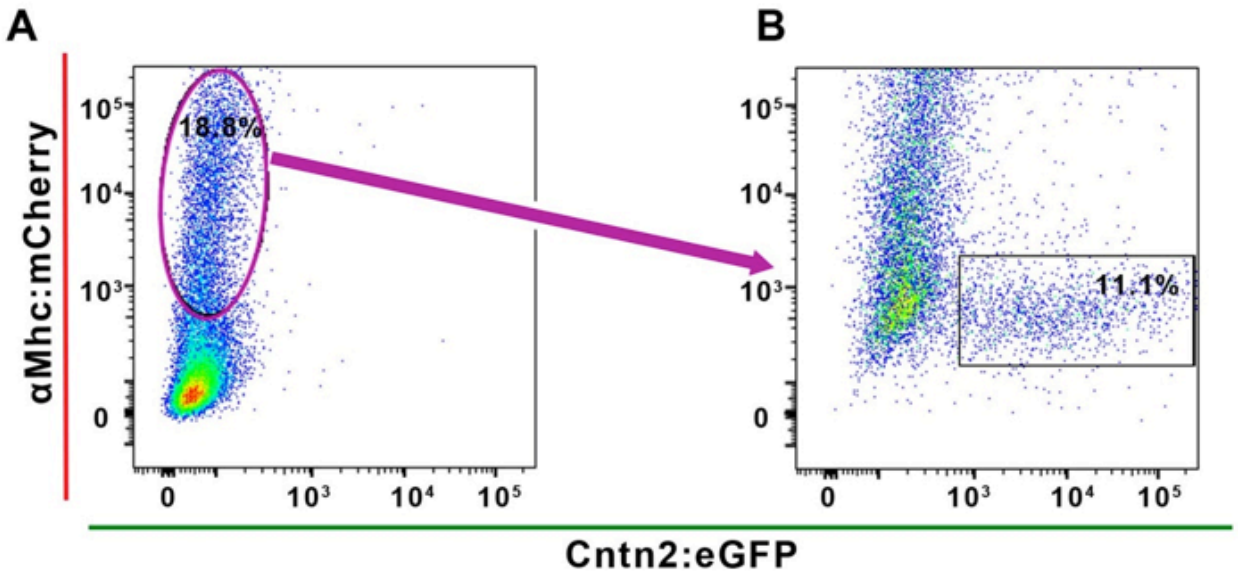
A) Quantification of immunofluorescence staining of HCN4$^+$, Cntn2:eGFP$^+$ and HCN4/Cntn2:eGFP double-positive cells by MetaExpress image analysis. The data are combined results from three independent differentiation experiments. Error bars are S.D. ** indicates $p<0.01$.

B) Expression of a well characterized neural marker, TUBB3, was examined by immunofluorescence staining. Triple-immunofluorescence staining of TUBB3, HCN4 and GFP in spontaneously differentiating cells is shown in the left panel. These cultures were used as a TUBB3 positive control sample. Co-immunofluorescence staining of GFP and HCN4 with TUBB3 in SN-treated cells is shown in the right panel. C) Quantification of transcript levels for neural genes *Mash1*, *Pax3*, *Sox3* and *Tubb3* were determined by qPCR.



Supplemental Figure S3. GFP+ cells isolated from DMSO-treated cells express PC expression profiles comparable to the SN-induced GFP+ cells.

Gene expression profiles of three cell populations isolated from DMSO-treated cells (negative, blue bars, normalized to 1; αMhc:mCherry^{+} (GFP-negative), red bars; Cntn2:eGFP^{+} (mCherry-dim), green bars) analyzed by qPCR. Comparison for significance of either mCherry^{+} or eGFP^{+} cell populations with the negative population is indicated by asterisks (*). Comparison of Cntn2:eGFP^{+} cells with αMhc:mCherry^{+} cells are shown by hashtags (#). Error bars show S.D. Results are from 3 independent experiments.

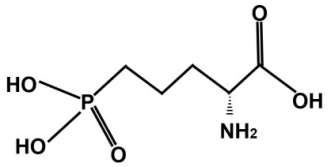
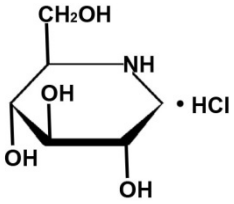
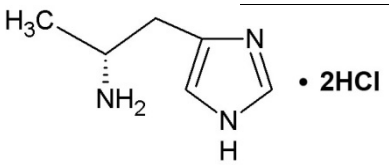
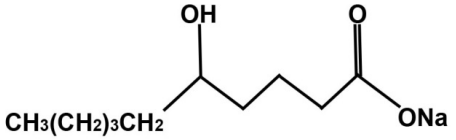
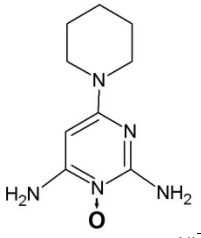
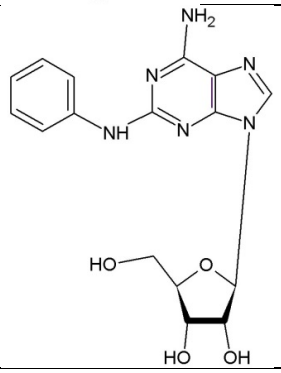


Supplemental Figure S4. Cntn2:eGFP^{+} cells are derived from αMhc:mCherry^{+} cells.

A) FACS plot of αMhc:mCherry/Cntn2:eGFP double reporter line at differentiation day 11. αMhc:mCherry^{+} cells were sorted and re-cultured. B) Flow plot of Cntn2:eGFP positive cells that were analyzed after 14 days culture of the sorted mCherry+ cells. Plot is representative of 3 reproducible independent experiments. Note that the double-positive cells are relatively mCherry^{dim}.

Table S1.

Supplement Table S1. Chemical structure of 12 additional hit compounds from the initial chemical screen that were “cherry-picked” but otherwise not further evaluated.

Name	Name of Library	Chemical Structure
D(−)-2-Amino-5-phosphonopentanoic acid NMDA receptor antagonist	LOPAC L19	
1-Deoxynojirimycin hydrochloride	LOPAC I10	
(R)(−)-α-Methylhistamine dihydrochloride	LOPAC L18	
5-Hydroxydecanoic acid sodium salt	LOPAC N18	
Minoxidil	LOPAC M14	
2-Phenylaminoadenosine	LOPAC C13	

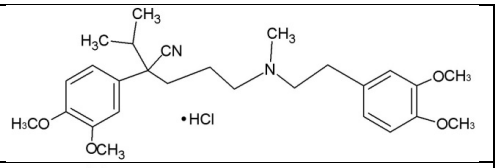
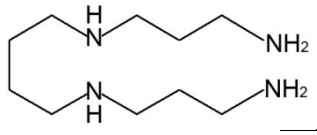
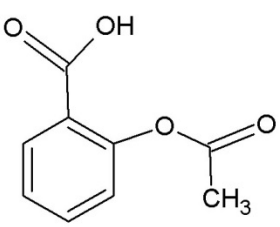
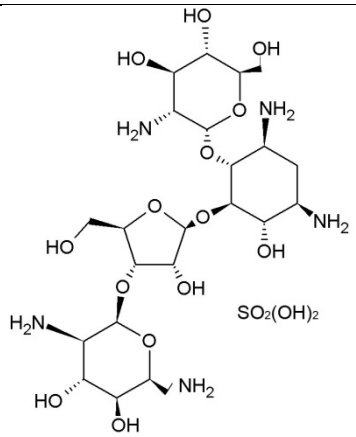
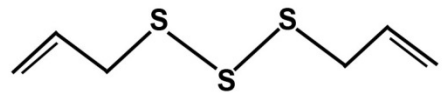
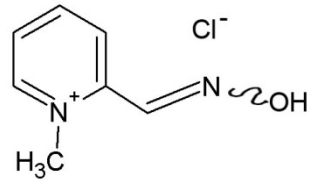
(±)-Verapamil hydrochloride	MicroSource D14	
Spermine	MicroSource E11	
Acetylsalicylic acid	LOPAC G17	
Paromomycin sulfate salt	LOPAC C11	
Diallyl Trisulfide	Prestwick D11	
Pyridine-2-aldoxime methochloride	Prestwick N8	

Table S2.

	GFP-, n=12	GFP+, n=8
Resting Potential, mV	-67.8±0.6	-69.2±1.0
Action Potential Amplitude, mV	114.5±3.0	122.2±1.9
APD_{50}, ms	127.3±26.8	315.2±53.7
APD_{90}, ms	196.1±30.9	395.9±54.2
dV/dtmax, V/s	82.5±12.9	122.6±13.9

Supplemental Table S2. Characterization of the action potential in ESC derivatives. Comparison of action potential (AP) properties (resting potential, action potential amplitude, maximum rate of rise of AP upstroke, APD_{50} and APD_{90}) between Cntn2:eGFP negative and positive cells.

Supplemental Table S3. List of qPCR primers

Gene	Forward primer(5' to 3')	Reverse primer (5' to 3')
Gapdh	CTAACATCAAATGGGGTGAGG	CGGAGATGATGACCCTTTTG
Tbx3	CCACCTCCAACAACACGTTCT	TAAGGAAACAGGCTCCCGAA
Tbx5	CAGGAGCACAGCCAAATTTAC	CCATGTACGGCTTCTTATAGGG
Tbx18	TGTCCCCCATCAAGCCTGTT	ATGGCCTCCAGAATGCGTATG
Id2	AGGTGACCAAGATGGAAATC	GCTCAGAAGGGAATTCAGAT
Nkx2.5	CATTTTACCCGGGAGCCTAC	CTTTGTCCAGCTCCACTGC
Gata6	TACACAAGCGACCACCTCAG	GTAGAGGCCGTCTTGACCTG
Hcn4	TCCTTGATCCCTTCAGCTCT	AGAGAATCCAGCCAGCTGTT
Cx30.2	GCAGGAGGAGTTCGTGTGTA	AGGATGTGGAAGAGCCAGA
Scn5a	GGGACTCATTGCCTACATGA	GCACTGGGAGGTTATCACTG
Scn10a	CTAGTCTGTTGTTTTCTGCG	GCGAAGAGCAGCGTGCGAATC
Cntn2	CATGTCTTCAGCCACTGACC	TGGCCTTGTCCTGGGTTAT
Cx40	GGAAGACGGGCTGTTCCA	CCCATTTCAGAAAACAAACACA
Tnnt2	CAGAGGAGGCCAACGTAGAAG	CTCCATCGGGGATCTTGGGT
Kir2.1	GTGGCTTGTGACCCTCTGTA	TCTCGACGCTTCTCTCTTGA
Cav1.3	GTTGTAAGTGCGGTAGAAAGCA	CTGGTGCCTCTTGCATAGTTT
Cav3.1	TAACCTGCTTGTCGCCATT	ACTCGTATCTTCCCGTTTGC
Mash1	ACTTGAACTCTATGGCGGGTT	CCAGTTGGTAAAGTCCAGCAG
Pax3	GCAGCGCAGGAGCAGAACCA	GCACTCGGGCCTCGGTAAGC
Sox3	AAGATGCACAACTCCGAGAT	GTACTTGTCCTTCTTGAGCAG
Tubb3	TGGCGCCTTTGGACACCTA	AAGCCGGGCATGAAGAAGTG

Supplemental Movie S1. Live imaging of αMhc:mCherry/Cntn2:eGFP double reporter line at differentiation day 12 to day 17.

As shown by live imaging, αMhc:mCherry^{+} cells can be seen at differentiation day 12. The first faint Cntn2:eGFP^{+} cells can initially be seen at this time and the signal co-localizes with αMhc:mCherry^{+} cells at 12 days and gradually increases in GFP intensity over 20 hr.