

Fig. S1

(A) IF expression of pluripotency markers OCT4 (red) co-localized with SOX2 (Green) or NANOG (green) in H1 hESC prior to differentiation to NCs. (B) Related to Figure 1A, expanded IF of PAX7 (red) and SOX10 (green) expression on day 5 in H1 hESCs at different doses of CHIR, with channels separated to reveal expression of individual markers. (C) IF expression on day 5 following 0-2 day treatment with either 3µM or 10µM CHIR for SOX10 (red), AP2A (aqua), and HNK1 (green) in columns 1, 2 and 3, respectively, and SOX10 (green) and SNAI2 (red) in columns 5 and 6. (D) IF expression of PAX7 (red) and SOX10 (green) on day 5 of hESCs treated with recombinant human, rhWNT3A, on days 0-2 at doses indicated. (E) IF expression of PAX7 (red) and SOX10 (green) on day 5 of hESCs following 0-2 day treatment with 10µM CHIR in either CONTROL scrambled siRNA (left), or β-CATENIN siRNA (right), and RT-qPCR for β-CATENIN and AXIN2. Error bars are +/- SEM and differences were evaluated by t-test, *** $P < 0.0005$. (F) IF merge of PAX7 (red) and SOX10 (green) on day 5 of hESCs treated with 0-2 day with either 3µM CHIR or 10µM CHIR. IF expression of HOXC9 (aqua) in these conditions are shown on right of views containing SOX10/PAX7 at corresponding fields. (G) RT-qPCR for thoracic through sacral level HOX genes, HOXC9, HOXA10, HOXA11, and HOXD13 on day 5, following 0-2 day treatment with 3µM or 10µM CHIR. Graph represents fold change levels vs ESC, and unpaired Student t-test was used to compare fold changes between the two doses, ** $P < 0.005$. Scale bars: 50µm in A, E; 225µm in B; 100µm in C, D; 300µm in F

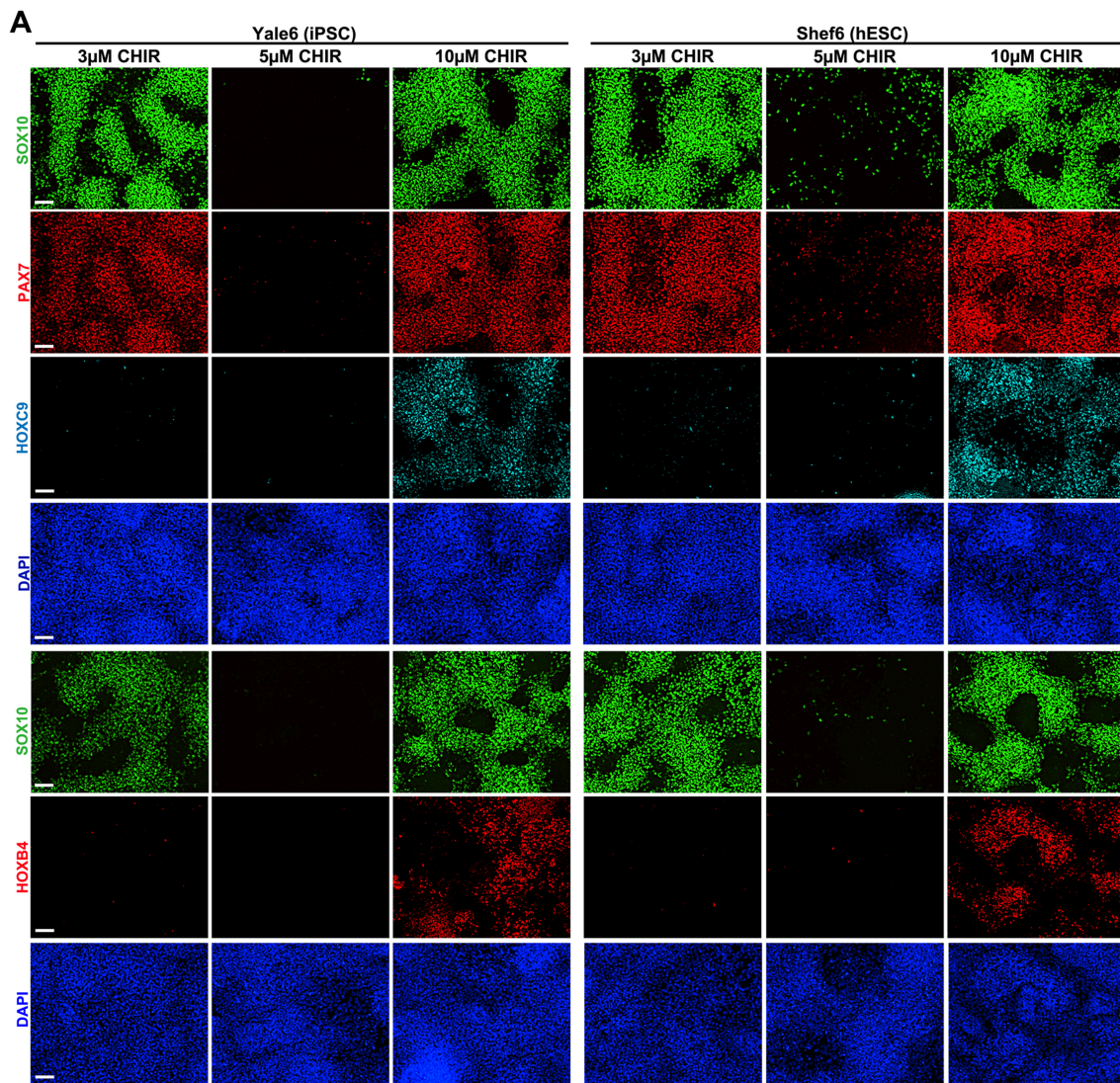


Fig. S2

(A) IF expression of PAX7 (red), SOX10 (green), HOXC9 (aqua) and DAPI (blue) in rows 1-4 respectively, of NCs on day 5 of culture for iPSC line, Yale 6 (columns 1-3) and hESC line, Shef6 (columns 4-6), following 0-2 day CHIR treatment at indicated doses. Rows 5-7 from top are IFs for SOX10 (green), HOXB4 (red) and DAPI (blue). Scale bar: 100 μ m

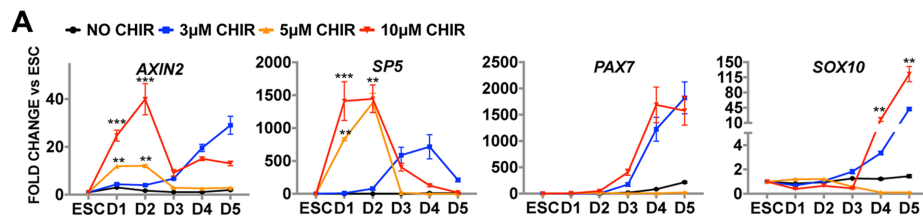


Fig. S3

(A) RT-qPCR expression levels of WNT/ β -CATENIN response targets *AXIN2* and *SP5* and NC markers *PAX7* and *SOX10* on a daily basis following a 0-2 day pulse with DMSO (NO CHIR), 3 μ M CHIR, 5 μ M CHIR, or 10 μ M CHIR. Fold change is relative to hESC and normalized by housekeeping genes. Error bars are \pm SEM. Unpaired Student t-test; each condition compares either 5 μ M or 10 μ M CHIR with 3 μ M CHIR * P <0.05 ** P <0.005 *** P <0.0005

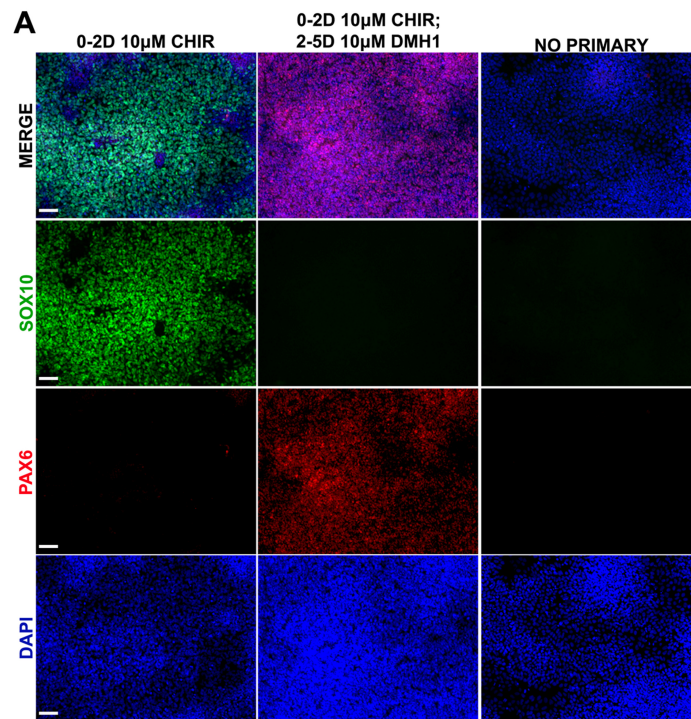


Fig. S4

(A) IF expression on day 5 for SOX10 (green) and PAX6 (red) in cells treated for 0-2 days with 10 μ M CHIR and DMSO vehicle on days 2-5 (column1), or 10 μ M DMH1 on days 2-5 (column2). No primary IF controls (right column) were used to establish baseline levels. Scale bar: 100 μ m

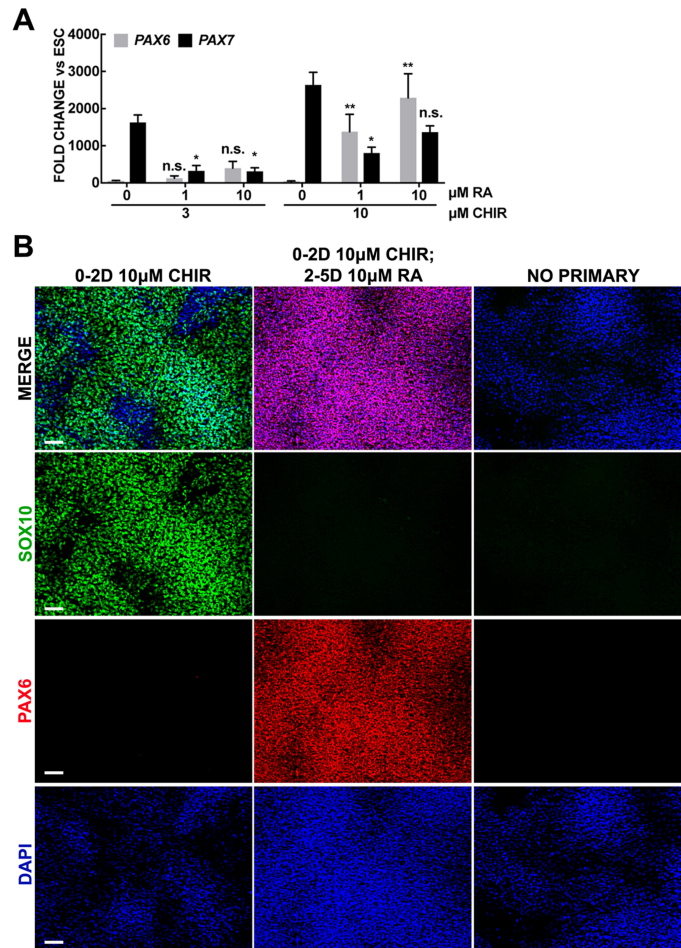


Fig. S5

(A) RT-qPCR on day 5 for *PAX6* and *PAX7* after 0-2 day treatment with 3μM or 10μM CHIR and either DMSO (0 RA), 1μM RA or 10μM RA on days 1-5. Error bars are +/- SEM, statistical significance was evaluated by t-test for each condition relative to either 3μM CHIR or 10μM CHIR * $P < 0.05$, ** $P < 0.005$. (B) IF expression on day 5 for SOX10 (green) and PAX6 (red) in cells treated for 0-2 days with 10μM CHIR and DMSO vehicle on days 2-5 (column1), or 10μM RA on days 2-5 (column2). No primary IF controls (right column) were used to establish baseline levels. Scale bar: 100μm

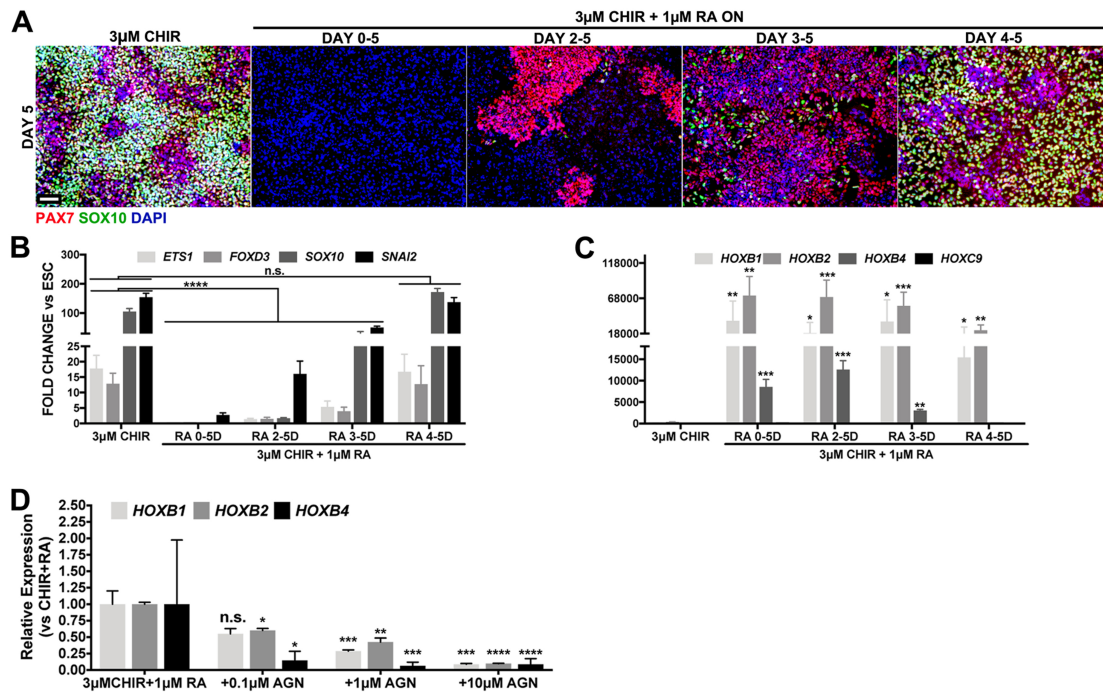


Fig. S6

(A) IF expression of PAX7 (red) and SOX10 (green) of cultures analyzed on day 5. All conditions received 3μM CHIR on days 0-2 and addition of 1μM RA on the days indicated above each image.

(B-C) RT-qPCR on day 5 treated cultures as indicated in panel A for NC markers, *ETS1*, *FOXD3*, *SOX10* and *SNAI2* (panel B) or *HOX* genes: *HOXB1*, *HOXB2*, *HOXB4*, and *HOXC9* (panel C). In both cases Fold Changes are relative to ESCs and normalized by housekeeping genes. Unpaired Student t-test are between RA treated conditions and untreated 3μM CHIR control, * p < 0.05, ** P < 0.005, *** P < 0.0005, **** P < 0.00005. (D) RT-qPCR on day 5 for *HOXB1*, *HOXB2* and *HOXB4* from cells treated with 3μM CHIR on 0-2 days followed by 1μM RA with days 4-5 (3μM CHIR+1μM RA) or 3μM CHIR followed by treatment on days 4-5 with 1μM RA and AGN193109 (AGN) at three different doses: (+0.1μM AGN), (+1μM AGN) or (+10μM AGN). Unpaired Student t-test between (3μM CHIR+1μM RA) and conditions supplemented with different doses of AGN, * p < 0.05, ** P < 0.005, *** P < 0.0005, **** P < 0.00005. Scale bar: 100μm

TABLE S1. Reverse Transcriptase quantitative PCR (RT-qPCR) primers

	FORWARD	REVERSE
<i>BACTIN</i>	TGAACCCCAAGGCCAACCGC	GACCCCGTCACCGGAGTCCA
<i>RPL27</i>	ATCGCCAAGAGATCAAAGATAA	TCTGAAGACATCCTTATTGACG
<i>SOX10</i>	CCTTCATGGTGTGGGCTC	CGCTTGTCACTTTCGTTTCCAG
<i>PAX7</i>	TGACAGCTTCATGAATCCGG	GATGGAGAAGTCAGCCTGTG
<i>PAX3</i>	GGCTTTCAACCATCTCATTCCCG	GTTGAGGTCTGTGAACGGTGCT
<i>FOXD3</i>	GCATCTGCGAGTTCATCAGC	CGTTGAGTGAGAGGTTGTGG
<i>MSX1</i>	GCTCGTCAAAGCCGAGAG	ACGGTTCGTCTTGTGTTTGC
<i>SNAI2</i>	CAGACCCTGGTTGCTTCAAG	GAGCCCTCAGATTTGACCTG
<i>ETS1</i>	GGAGATGGCTGGGAATTCAAAC	ACGGCTCAGTTTCTCATAATTCATC
<i>OCT4</i>	GAGAAGGAGAAGCTGGAGCA	CTTCTGCTTCAGGAGCTTGG
<i>NANOG</i>	GATTTGTGGGCCTGAAGAAA	CAGATCCATGGAGGAAGGAA
<i>KLF4</i>	ACCCACACAGGTGAGAAACC	ATGCTCGGTGCGATTTTTGG
<i>SOX2</i>	TCAAGCGGCCCATGAATGCC	AGCCGCTTAGCCTCGTCGAT
<i>AXIN2</i>	CGGGAGCCACACCCTTCT	TGGACACCTGCCAGTTTCTTT
<i>SP5</i>	CTTCGGGTGTCCATGCCTC	GTGCGGTCCTGGAGAAAGG
<i>ZIC1</i>	GTCCTACACGCATCCAGTT	GCGATAAGGAGCTTGTGGTC
<i>OTX2</i>	GAGAGGAGGTGGCACTGAAAA	GTTGTTGGCGGCACTTAGC
<i>DMBX1</i>	ATGTGGTGATGCGTGAGAGG	GCTGTTCTTCTGCAGGCTA
<i>HOXB1</i>	GTTAAGAGAAACCCACCCAAGAC	GGAATCCTTTTCCAGTTCTGTC
<i>HOXB2</i>	GCAGTCCCAGGCCATCTG	CGCCACGTCTCCTTCTCC
<i>HOXB4</i>	TACCCCTGGATGCGCAAAGTTC	TGGTGTGGGCAACTTGTGG
<i>HOXC5</i>	ACAGATTTACCCGTGGATGAC	AGTGAGGTAGCGGTTAAAGTG
<i>HOXC9</i>	AGCACAAAGAGGAGAAGGC	CGTCTGGTACTTGGTGTAGG
<i>HOXA10</i>	GGATTCCCTGGGCAATTCCAAA	CAGTGTCTGGTGCTTCGTGT
<i>HOXA11</i>	GGCGGCTCCAGTGGC	CGCTGAAGAAGAACTCCCCT
<i>HOXD13</i>	ACGCTGGCTAACGGGTG	TTAGAGCCACATCCCCTGGA
<i>EGR2</i>	TTTGACCAGATGAACGGAGTG	GCCCATGTAAGTGAAGGTCTG
<i>CDX1</i>	CACAATCCGGCGGAAATCAG	TTCTTGTTCACTTTGCGCTCC
<i>CDX2</i>	GGAACCTGTGCGAGTGGAT	TGAAACTCCTTCTCCAGCTCC
<i>TBXT</i>	CCTTCAGCAAAGTCAAGCTCACC	TGAACTGGGTCTCAGGGAAGCA
<i>MSGN1</i>	AACCTGCGCGAGACTTTCC	ACAGCTGGACAGGGAGAAGA
<i>TBX6</i>	TGCGGCAGCCTGTGTCT	GTGCATGGAGTGCAAGGATCA
<i>DUSP6</i>	AGCCAAGCAATGTACCAAGACA	CAGTTTTTCCCTGAGGCCATTTT
<i>SRY2</i>	GTCACTCCAGCAGGCTTAGAA	GCACATCGCAGAAAGAAGAGAAT
<i>NKX1-2</i>	TCCGCATCCTCCTCCTCTTC	GAAAGATGCCAGCTCCAGGG
<i>FGF8</i>	GCAGCGCTGTGTAGTTGTTT	ACAAGAAGGGGAAGCTGATCG
<i>PAX6</i>	CACCTACAGCGCTCTGCCGC	CCCGAGGTGCCATTGGCTG
<i>CYP26A1</i>	TTCGACTGAATCCCCAGTTC	TGTAGATAACATTCCAGCCCTTGG
<i>CRABP2</i>	TACCTGTGGCCACTCACTCT	TGACCAACGATGGGGAAGTCTG
<i>β-CATENIN</i>	CACAAGCAGAGTGCTGAAGGTG	GATTCCTGAGAGTCCAAAGACAG