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Supplemental Information

Deriving Dorsal Spinal Sensory Interneurons from Human Pluripotent Stem Cells

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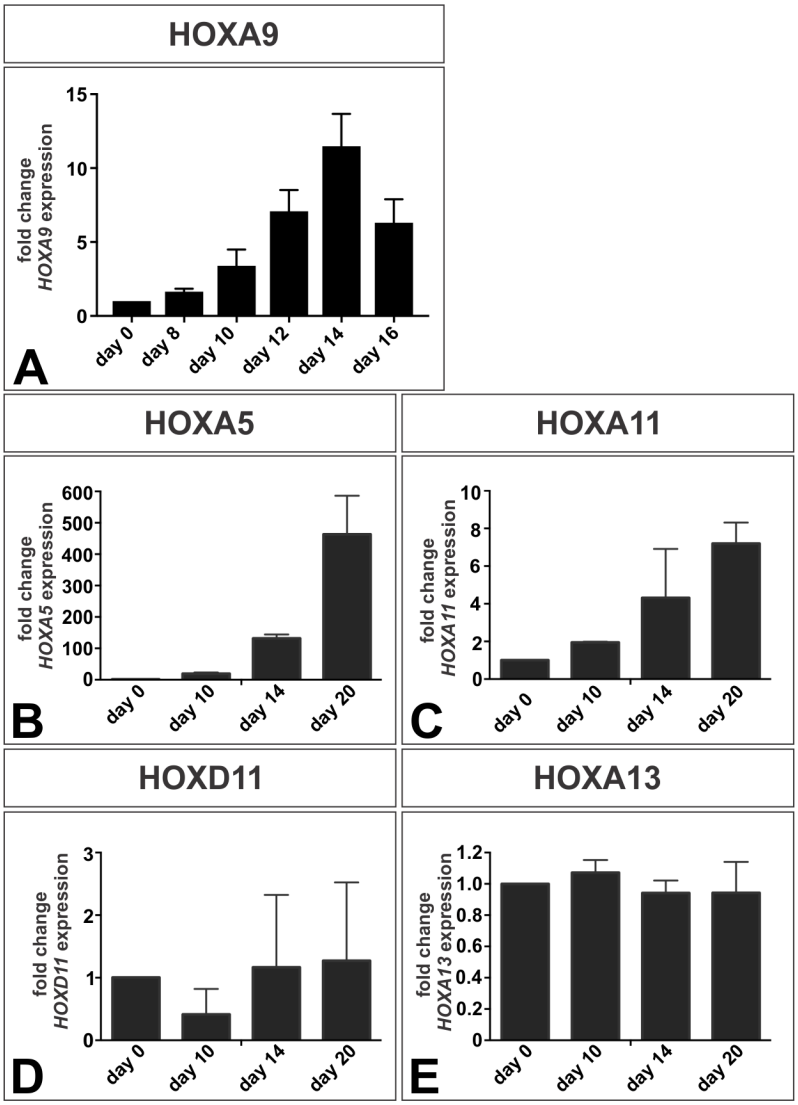
Supplementary information

Supplementary Figure 1: Retinoic acid induces a cervical HOX profile in hEBs

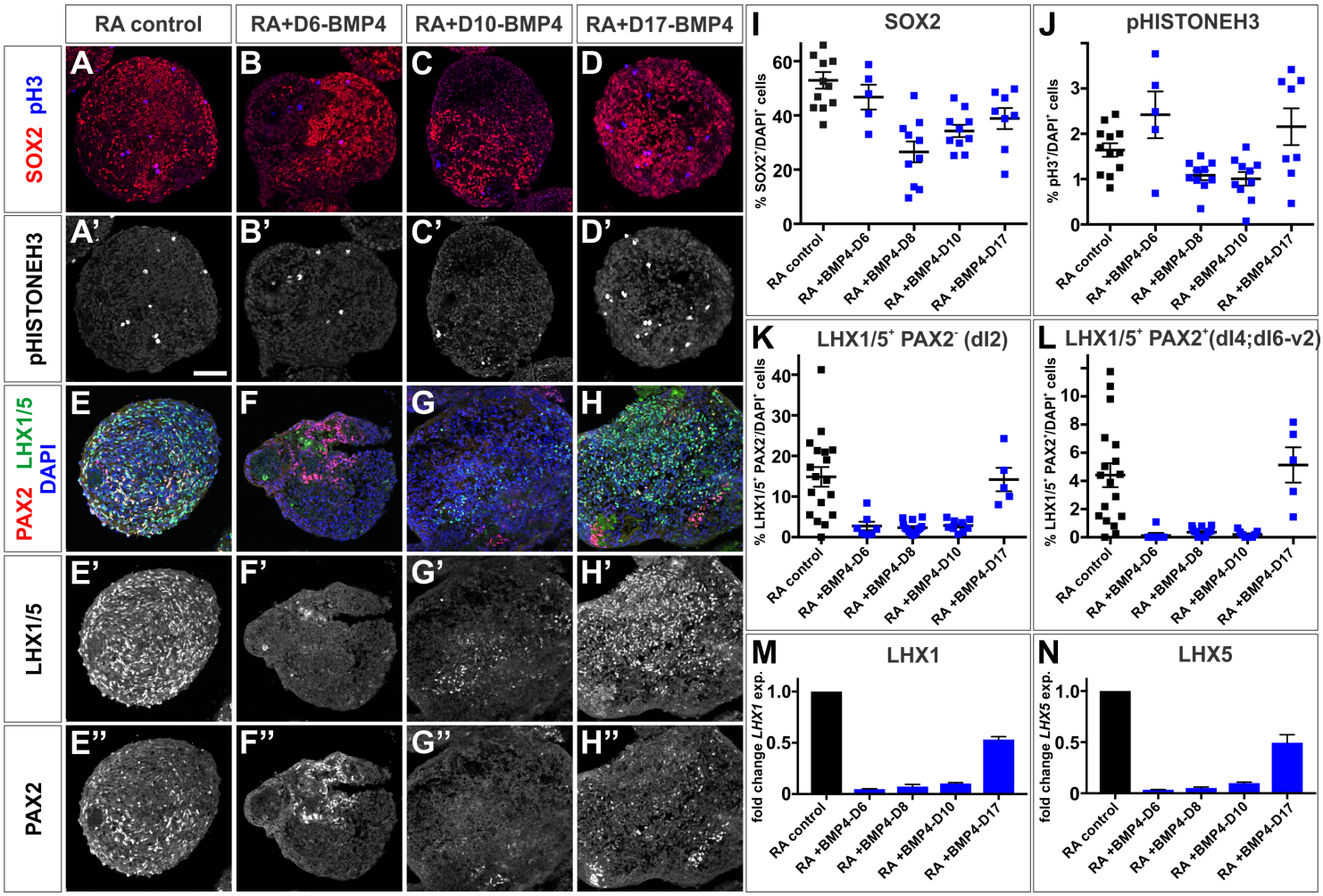


(A-E) RA-treated hEBs were assessed for the levels of *HOX* gene expression by RT-qPCR, which was normalized to day 0. By day 14-20 in the hESC differentiation protocol, there was significant increase in *HOXA9* (lower thoracic identity A, $p<0.04$, day 0 is similar to day 14), *HOXA5* (cervical level, B, $p<0.030$, day 0 versus day 20), and *HOXA11* (mid-lumbar level, C, $p<0.03$, day 0 versus day 20) expression in the hESC-derived EBs. There were no significant

changes in *HOXD11* (lower lumbar level, D, $p>0.84$, day 0 versus day 20) and HOXA13 (sacral level, E, $p>0.24$ for day 0 versus day 20).

Data are represented as mean±SEM. Two biological replicates were performed; the qRT-PCR conditions were run in triplicate.

Supplementary Figure 2: BMP4 suppresses the dI2 fate in hEBs.



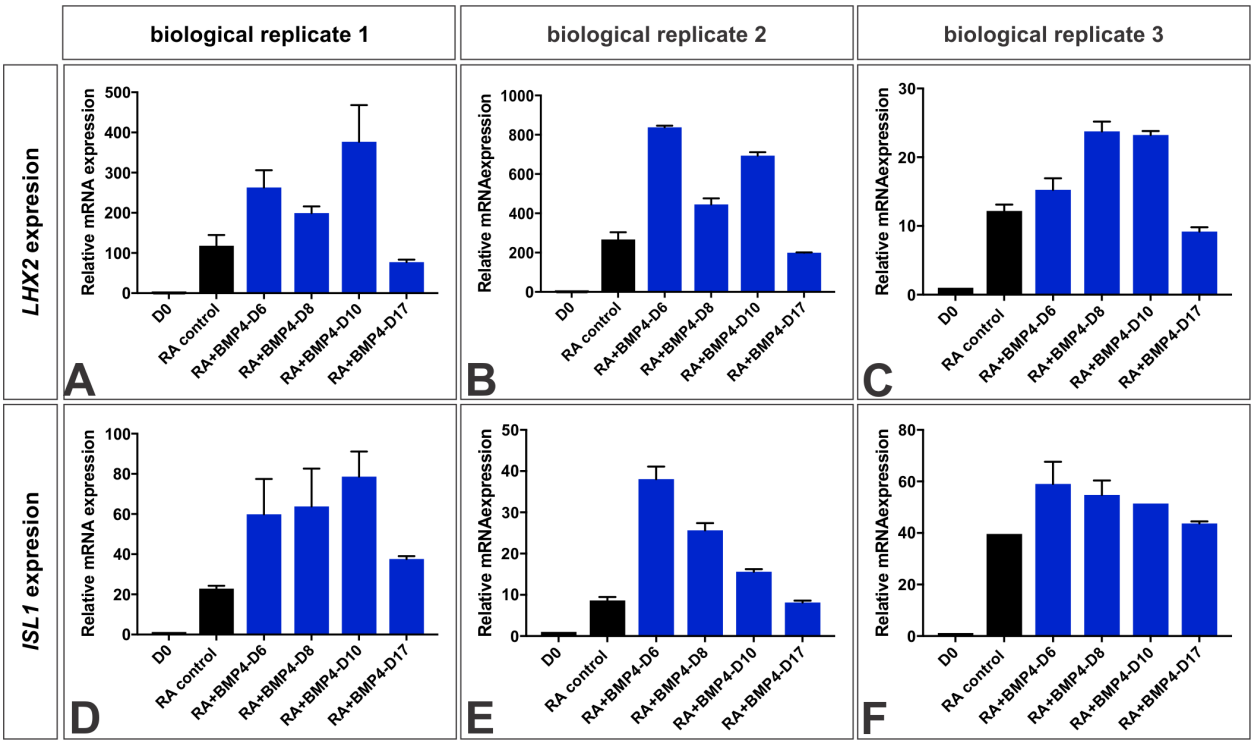
(A-D, I, J) By day 36 of the protocol, RA-treated hEBs still contain significant numbers of SOX2^{+} progenitors and phospho-HISTONEH3^{+} mitotic cells. The addition of BMP4, from day 8 on, significantly decreases the number of progenitors and dividing cells in hEBs (I, J; probability similar to RA control: $p<0.0001$, BMP4-D8; $p<0.0003$, BMP4-D10; $p<0.016$, BMP4-D17).

(E-H, K-N) RA treatment results in the generation of numerous LHX1/5^{+} PAX2^{-} cells (dI2) (E, E', K) and a few LHX1/5^{+} PAX2^{+} cells (dI4; dI6-v2) (E, E'', L). However, the specification of these cell types is suppressed by addition of BMP4 between day 6 and day 10 (dI2: F-G', K, $p<0.0015$, BMP4-D6; $p<0.0001$, BMP4-D8; $p<0.0005$, BMP4-D10; dI4, dI6-v2: $p<0.0001$ for all conditions) in hESCs derived EBs. By day 17, the addition of BMP4 no longer suppresses

dI2 identity (H-H", K; $p>0.9$, L; $p>0.48$). The effect of BMP4 on dI2 fates was also observed in an RT-qPCR analysis of *LHX1* and *LHX5* expression (M, N).

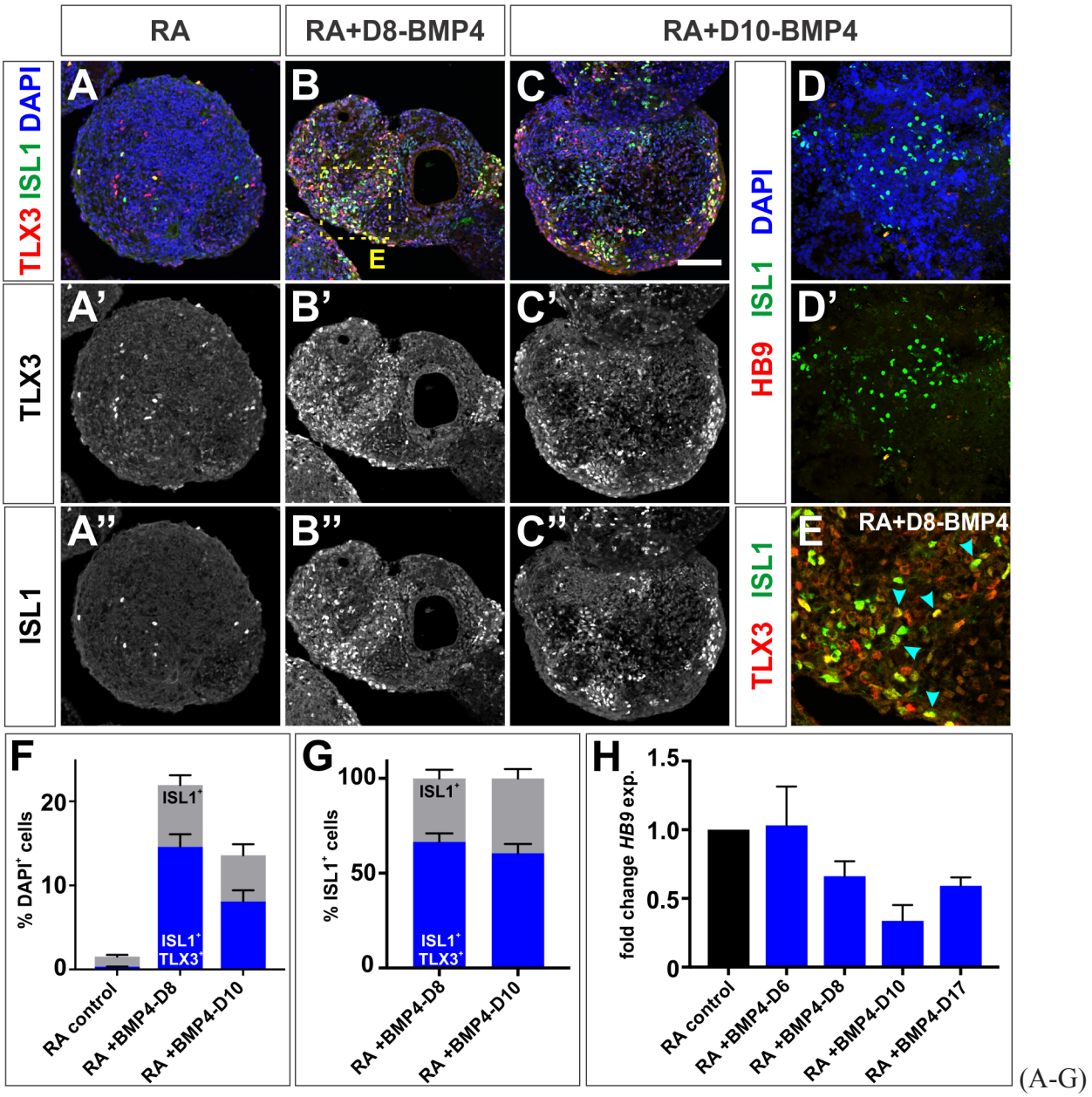
Data are represented as mean ± SEM. Two biological replicates were performed; the qRT-PCR conditions were run in triplicate and between 9-18 EBs were quantified for each condition, normalized to the total number of $DAPI^+$ cells and represented as the percent cell numbers. Scale bar=100 μm.

Supplementary Figure 3: Biological replicates for upregulation of LHX2 and ISL1 mRNA in the directed differentiation protocol



(A-F) RT-qPCR analysis of *LHX2* (A-C) and *ISL1* (D-F) expression in three biological replicates of the directed differention protocol. EBs were treated with RA, with BMP4 added at day 6, 8, 10 and 17. In each biological replicate *LHX2* and *ISL1* expression is upregulated after addition of BMP4, however there are marked differences in the magnitude of relative fold change. Three technical replicates were performed. Values are represented as mean±SEM.

Supplementary Figure 4: Characterization of hESC-derived mechanosensory dI3 cells



(A-G)

By day 36, BMP4 addition significantly increases the number of ISL1$^+$ TLX3$^+$ dI3 INs in the BMP4-D8 (B, E, $p<0.0001$ similar to RA control) and BMP4-D10 (C, $p<0.0001$) conditions compared to RA controls (A, F,). ~60% of dI3s are double positive for ISL1 and TLX3 (G). In contrast, the ISL1$^+$ cells are not co-labeled by the spinal motor neuron marker HB9 (D) and a RT-qPCR analysis showed that there was no *HB9* mRNA enrichment in any of the BMP4 conditions (H). Thus, the hESC-derived ISL1$^+$ cells no not have a motor neuron identity.

Data are represented as mean ± SEM. Two biological replicates were performed; the qRT-PCR conditions were run in triplicate and between 7-10 EBs were quantified for each condition. The number of $ISL1^+$ cells and $ISL1^+$ $TLX3^+$ cells were normalized to the number of $DAPI^+$ cells in D and to the total $ISL1^+$ cells in E.

Scale bar=100μm.

Table 1: Primary Antibodies

Antigen	Species	Dilution	Source
Sox2	Goat	1:1000	Santa Cruz Biotechnology (sc-17320)
Pax3	Goat	1:500	R&D Systems (AF2457)
Lhx2	Goat	1:250	Santa Cruz Biotechnology (sc-19344)
Isl1	Goat	1:500	R&D Systems (AF1837)
Sox1	Goat	1:500	Santa Cruz Biotechnology (sc-17318)
Pax6	Mouse	1:100	Developmental studies Hybridoma Bank (AB528427)
Nanog	Rabbit	1:200	Cell Signaling Technology (D73G4)
HoxA5	Goat	1:1000	Santa Cruz Biotechnology (sc-13199)
Olig2	Rabbit	1:300	Milipore (AB9610)
Tuj1	Mouse	1:1000	BioLegend (801202)
Dcc	Goat	1:200	R&D System (AF844)
Robo3	Goat	1:200	R&D System (AF3076)
Tlx3	Guinea pig	1:200	gift from Thomas Müller (Muller et al., 2005)
Lhx1/5	Mouse	1:20	Developmental Studies Hybridoma Bank
Pax2	Rabbit	1:500	Invitrogen

Table 2: RT-qPCR primer sequences

Gene name	Primer sequence
Nanog	For: CCCCAGCCTTTACTCTTCCTA Rev: CCAGGTTGAATTGTTCCAGGTC

Sox2	For: CAAAGAAAAACGAGGGAAATGGG Rev: TACCGGGTTTTCTCCATGCTG
Sox1	For: GCGGTAACAACTACAAAAAACTTGTAA Rev: GCGGAGCTCGTCGCATT
Pax6	For TTGAGCCATCACCAATCAGC Rev: TTTCTCCACGGATGTTGCTG
TUBB3 (β-III tubulin)	For: GGCCAAGGGTCACTACACG Rev: GCAGTCGCAGTTTTCACACTC
HoxA5	For: AAGTCATGACAACATAGGCGG Rev: TTCAATCCTCCTTCTGCGGG
Pax3	For: AGCACTGTACACCAAAGCAC Rev: AAAATCCATGCCTGGTGCTG
Olig2	For: CCCTAAAGGTGCGGATGCTT Rev: ACCCGAAAATCTGGATGCGA
Lhx2	For: TGGACCGAGGAACAACTTGG Rev: TCGCTCAGTCCACAAAACTG
Isl1	For: GATTTGGAATGGCATGCGGC Rev: GCGCATTTGATCCCGTACAA
Atoh1	For: ACCAGCTGCGCAATGTTATC Rev: TTTGTAGCAGCTCGGACAAG
Ascl1	For: AGCTTCTCGACTTCACCAACTG Rev: TGCTTCCAAAGTCCATTCGC
FoxD3	For: CGGCCTCGAGCAACAAATG Rev: AAATTGGGGAGAGGCAGAGTC
Lhx1	For: CAACATGCGCGTCATTCAGG Rev: ACTCGCTCTGGTAATCTCCG
Lhx5	For: GCGTCATCCAGGTGTGGTTT Rev: GGTGGACCCCAACATCTCAG

HoxA9	For: GTCCCACGCTTGACACTCA Rev: GCTGCTGGGTTATTGGGATCG
HoxD11	For: CAGCAGCGCAGTTGCC Rev: CGGTCAGTGAGGTTGAGCAT
HoxA13	For: CTGCCCTATGGCTACTTCGG Rev: CCGGCGGTATCCATGTACT
HoxA11	For: CCCGCAGTCTCGTCCAATTT Rev: AGGCTGTCTCGAAAAACTGGT

Table 3. Summary of the differentiation efficiencies.

	SOX2 (progenitor)	LHX2 (dI1)	LHX1/5^{+} PAX2^{-} (dI2)	ISL1 (dI3)	LHX1/5^{+} PAX2^{+} (dI4; dI6-v2)
RA	53%	8.5%	15%	1.5%	4.5%
RA+BMP4-D6	47%	26%	3%	15%	<0.2%
RA+BMP4-D8	26.5%	20%	2%	14.5%	<0.5%
RA+BMP4-D10	34%	18.5%	3%	8%	<0.2%
RA+BMP4-D17	39%	8%	14%	2%	5%