

## Supplementary Materials

**Table S1.** Table of Antibodies.

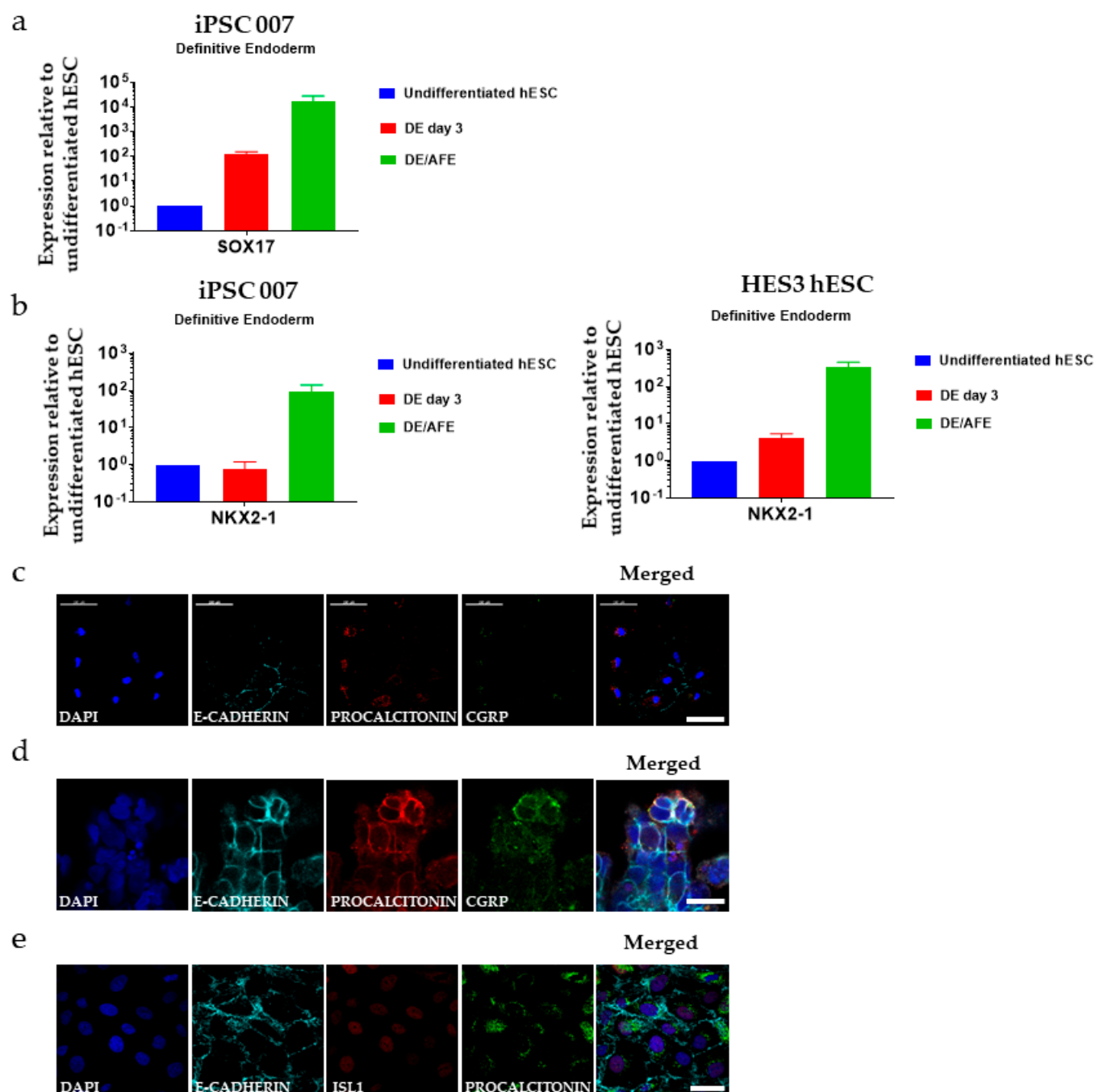
Primary Antibodies	Target Type	Host/Type	Company	Dilution
Anti-Procalcitonin (ab166963)	C cells	Sheep polyclonal	Abcam	1:200
Anti-Calcitonin (A0576)	C cells	Rabbit polyclonal	Dako	1:400–800
Anti-FoxA2 [EPR4466] (ab108422)	DE/C cells	Rabbit monoclonal	Abcam	1:300
Anti-MASH1/Achaete-scute homolog 1 antibody (ab38556)	Neuronal	Rabbit polyclonal	Abcam	1:500
Anti-PGP9.5 (ab72910)	Neuronal	Chick Polyclonal	Abcam	1:500
Anti-Beta III Tubulin raised in chicken	Neuronal	Chick Polyclonal	MERCK PL	1:1000
Anti-E-cadherin (610181)	Epithelial	Mouse IgG2a	BD Biosciences	1:200–1000
Recombinant Anti-TTF1 antibody [EP1584Y] (ab76013)	Thyroid/C cells	Rabbit monoclonal	Abcam	
Secondary Antibodies		Host/Isotype	Company	Dilution
Anti-Sheep IgG:Alexa 488 (A-11015)		Donkey	ThermoFisher Sci.	1:1000
Anti-Sheep IgG:Alexa 594 (A-11016)		Donkey	ThermoFisher Sci.	1:1000
Anti-Rabbit IgG Alexa 488 (A-21206)		Donkey	ThermoFisher Sci.	1:1000
Anti-Rabbit IgG Alexa 594 (A-21207)		Donkey	ThermoFisher Sci.	1:1000
Anti-Rabbit IgG Alexa 633 (A-21070)		Goat	ThermoFisher Sci.	1:1000
Anti-Mouse IgG-specific:Alexa 488 (A-11001)		Goat	ThermoFisher Sci.	1:1000
Anti-Mouse IgG-specific:Alexa 488 (A-21202)		Donkey	ThermoFisher Sci.	1:1000
Anti-Mouse IgG+M Alexa 594 (A21203)		Donkey	ThermoFisher Sci.	1:1000
Anti-Mouse IgG Alexa 647 (A-31571)		Donkey	ThermoFisher Sci.	1:1000
Anti-Chick IgY Alexa 488 (703-545-155)		Donkey	Jackson Immuno-research	1:400–800
Anti-Chick IgY Alexa 568 (A-11041)		Goat	ThermoFisher Sci.	1:1000
Anti-Chick IgY Alexa 488 (A-11039)		Goat	ThermoFisher Sci.	1:1000

**Table S2.** qPCR probes.

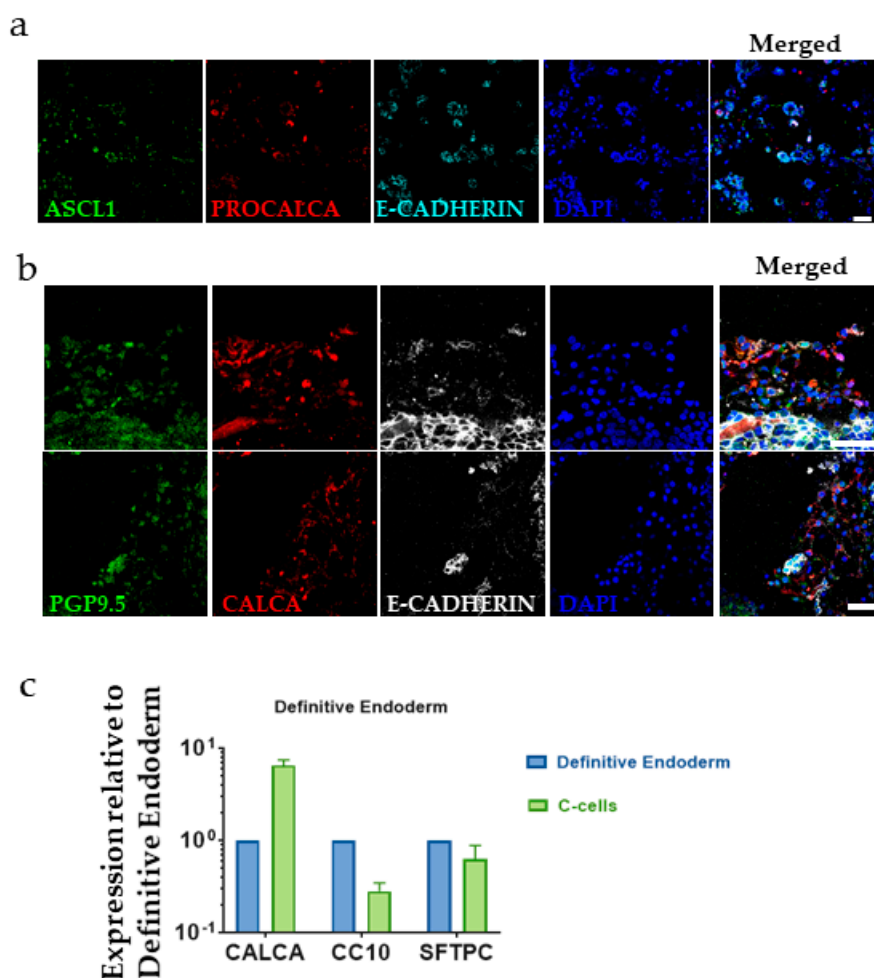
Probes	Target Type	Product Size	Company	Accession Number
<i>CDH1</i> (E-cadherin)	Cell-to-cell adhesion protein	80	ThermoFisher Sci	Hs01023895_m1
<i>CDH2</i> (N-cadherin)	Cell-to-cell adhesion protein	66	ThermoFisher Sci	Hs00983056_m1
<i>FOXA1</i>	Endodermal Marker	59	ThermoFisher Sci	Hs04187555_m1
<i>FOXA2</i>	DE Marker	66	ThermoFisher Sci	Hs00232764_m1
<i>RET</i>	Growth factor receptor	83	ThermoFisher Sci	Hs01120030_m1
<i>GAPDH</i>	Housekeeper	93	ThermoFisher Sci.	Hs02758991_g1

Table S3. PCR primers for SYBR green qPCR.

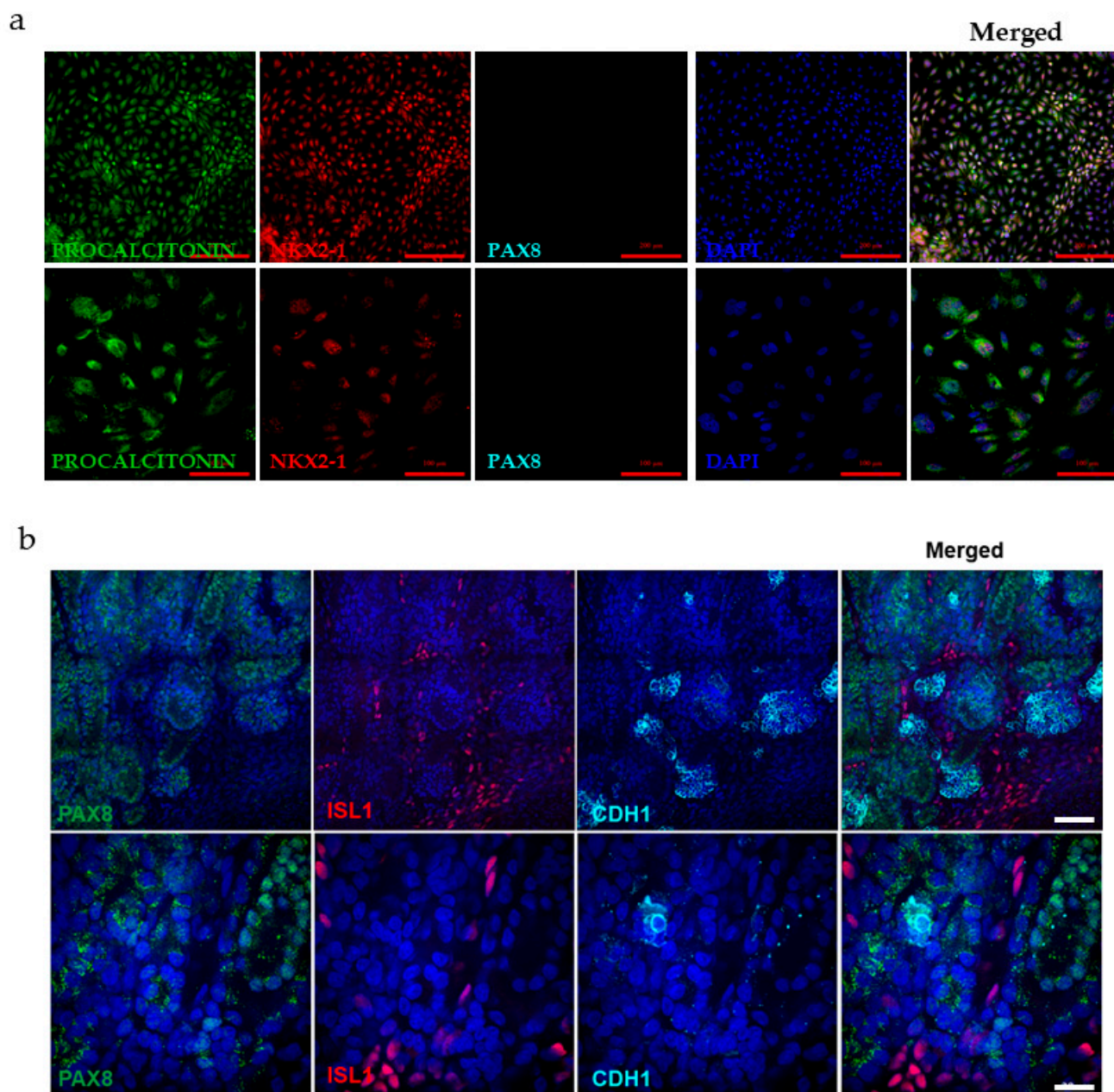
Gene	Target Type	Sequence 5' to 3'	Product Size
<i>GAPDH</i>	Housekeeper	F: GTCTCCTCTGACTTCAACAGCG R: ACCACCCTGTTGCTGTAGCCAA	131 bp
<i>TG</i>	Thyroid cell marker	F: CCAGTGGCTTCTCTTCCTGACT R: CCTTGGAGGAAGCGGATGGTTT	155 bp
<i>TSHR</i>	Thyroid Cell marker	F: GAGTTTCCTTCACCTCACACGG R: CTGCTCTCATTACACATCAAGGAC	114 bp
<i>RET</i>	Growth factor receptor	F: GAGGAGAGACTACTTGGACCTTG R: GGGGACAGCGGTGCTAGAAT	200 bp
<i>EPHA4</i>	Growth factor receptor	F: TTCTGCTATCTTGGCCTCACAG R: TAGACGGAAGTGAAGGAGGT	85 bp
<i>CDX2</i>	Posterior endoderm/Intestinal differentiation	F: ACAGTCGCTACATCACCATCCG R: CCTCTCCTTTGCTCTGCGGTTC	107 bp
<i>NKX2.1</i>	DE Marker/Thyroid Transcription Factor	F: GCAACCTGGGCAACATGAG R: CATGAAGCGGGAGATGGCG	122 bp
<i>CER1</i>	DE marker	F: CAGGACAGTGCCCTTCAGCCA R: ACAGTGAGAGCAGGAGGTATGG	142 bp
<i>CALCA</i>	C-cell Marker	F: GTCATGGGCTTCCAAAAGTTC R: CAGGGCAGACCTGAATGGTG	99 bp
<i>PAX8</i>	Thyroid Follicular Marker	F: CGATGCCTCACAACTCCATCA R: GCCAGGTCTACGATGCGCT	115 bp
<i>FOXE1</i>	Thyroid differentiation marker	F: CTCAAACGGGATGCTTTCTGGT R: GCACATGGAAGGCTGAAACTGA	126 bp



**Figure S1.** (a) QPCR analysis of DE/AFE-like cells normalized to undifferentiated hiPSC (007 Cell line), showing time course upregulation of *SOX17*. No significant difference was recorded. N.D.- Not Detected.  $n = 3$  independent experiments. Error bars represent mean  $\pm$  SEM. (b) QPCR analysis of DE/AFE-like cells normalized to undifferentiated hiPSC (007 Cell line) and hESC (HES3), showing time course upregulation of *NKX2.1*. No significant difference was recorded. N.D.- Not Detected.  $n = 3$  independent experiments. Error bars represent mean  $\pm$  SEM. (c) Immunofluorescence of differentiated thyroid C cell-like cells grown on laminin showing co-expression of C cell lineage markers, E-CADHERIN, CGRP and PROCALCITONIN. Scale bar: 100  $\mu$ m. (d) Immunofluorescence of differentiated thyroid C cell-like cells grown on Matrigel showing co-expression of C cell lineage markers, E-CADHERIN, CGRP and PROCALCITONIN. Scale bar: 20  $\mu$ m. (e) Immunofluorescence of differentiated thyroid C cell-like cells grown on Matrigel showing co-expression of C cell lineage markers, E-CADHERIN, ISL1 and PROCALCITONIN. Scale bar: 20  $\mu$ m.



**Figure S2.** (a) Wholemout staining of differentiated thyroid C cell-like cells in 3D Matrigel culTable 1. Scale bar: 100  $\mu$ m. (b) Wholemout staining of differentiated thyroid C cell-like cells in 3D Matrigel co-expressing PGP9.5, E-CADHERIN and CALCITONIN. Scale bar: 100  $\mu$ m. (c) QPCR analysis of thyroid C cell-like cells normalized to differentiated DE/AFE (007 Cell line), showing the downregulation of lung markers- *CC10* and *SFTPC* and upregulation of thyroid C cell-like cells-*CALCA*. No significant difference was recorded. N.D.- Not Detected.  $n = 3$  independent experiments. Error bars represent mean  $\pm$  SEM.



**Figure S3.** (a) Immunofluorescence of differentiated thyroid C cell-like cells grown on Laminin showing co-expression of C cell lineage markers, *NKX2.1* and *PROCALCITONIN* and no expression of thyroid marker, *PAX8*. Scale bar: 200  $\mu$ m (upper) and 100  $\mu$ m (lower). (b) Immunofluorescence of differentiated kidney cells as a positive control showing the specificity of the *PAX8* antibody. Scale bar: 200  $\mu$ m (upper) and 100  $\mu$ m (lower).