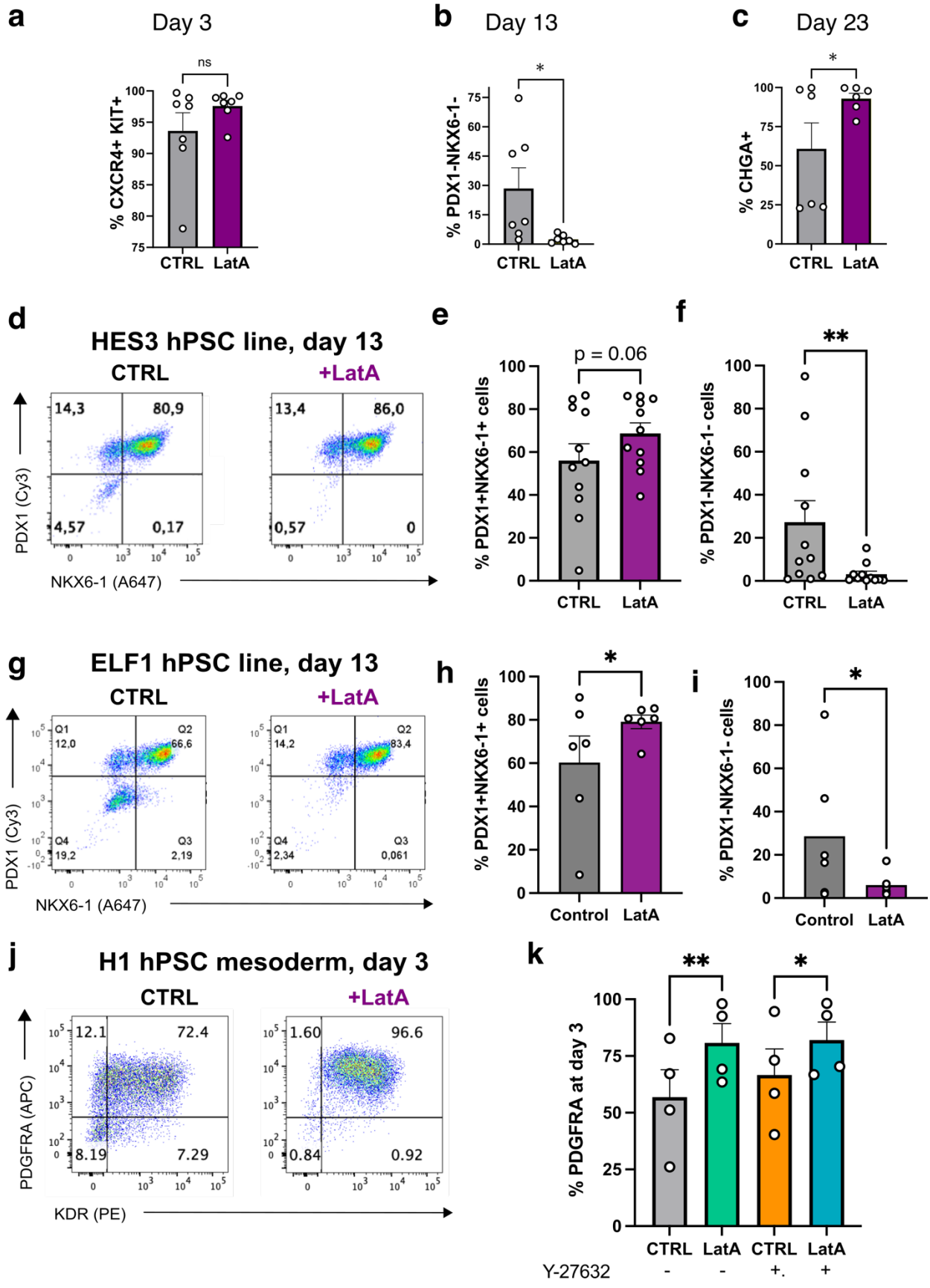
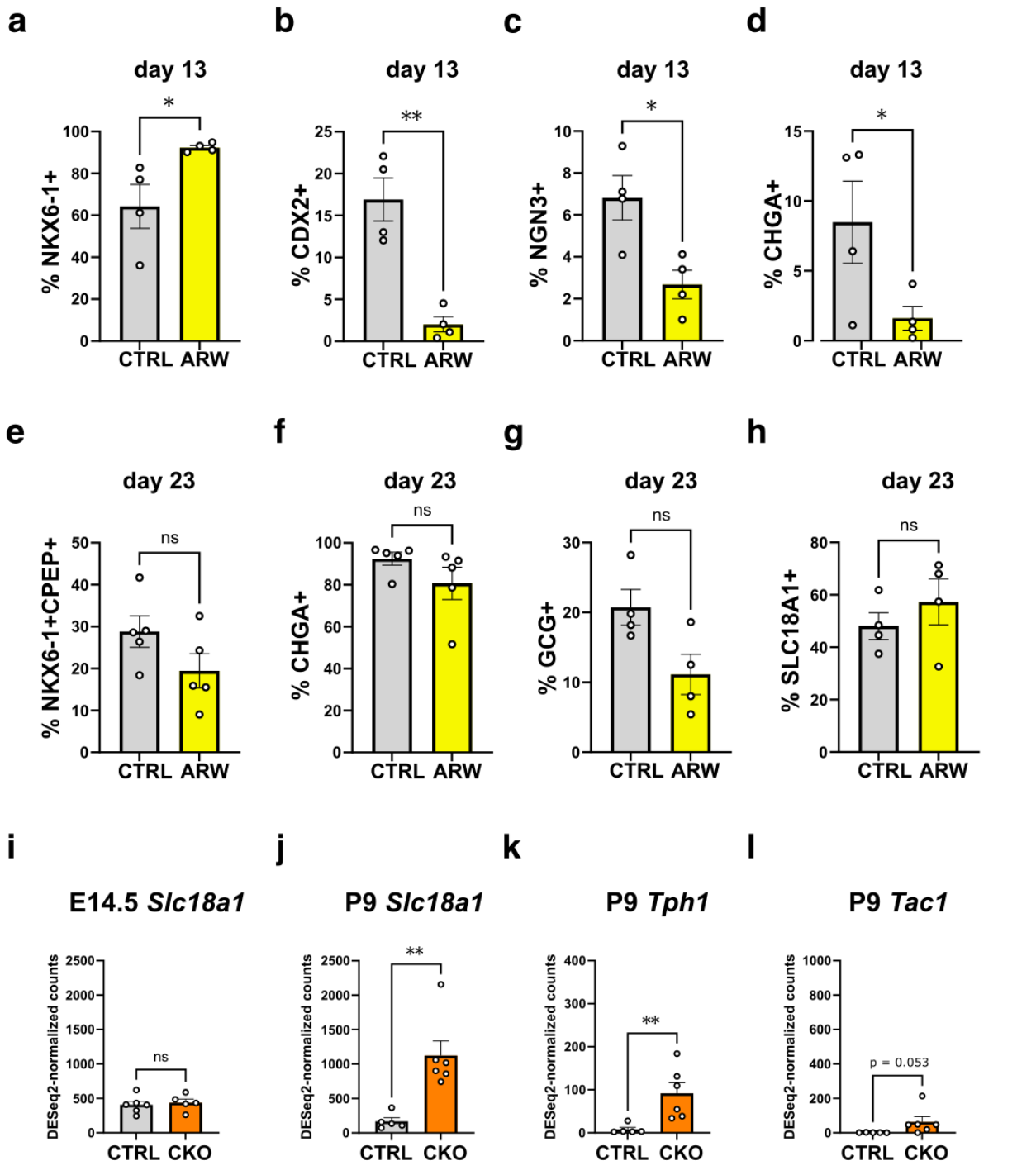
Supplemental Figure 1



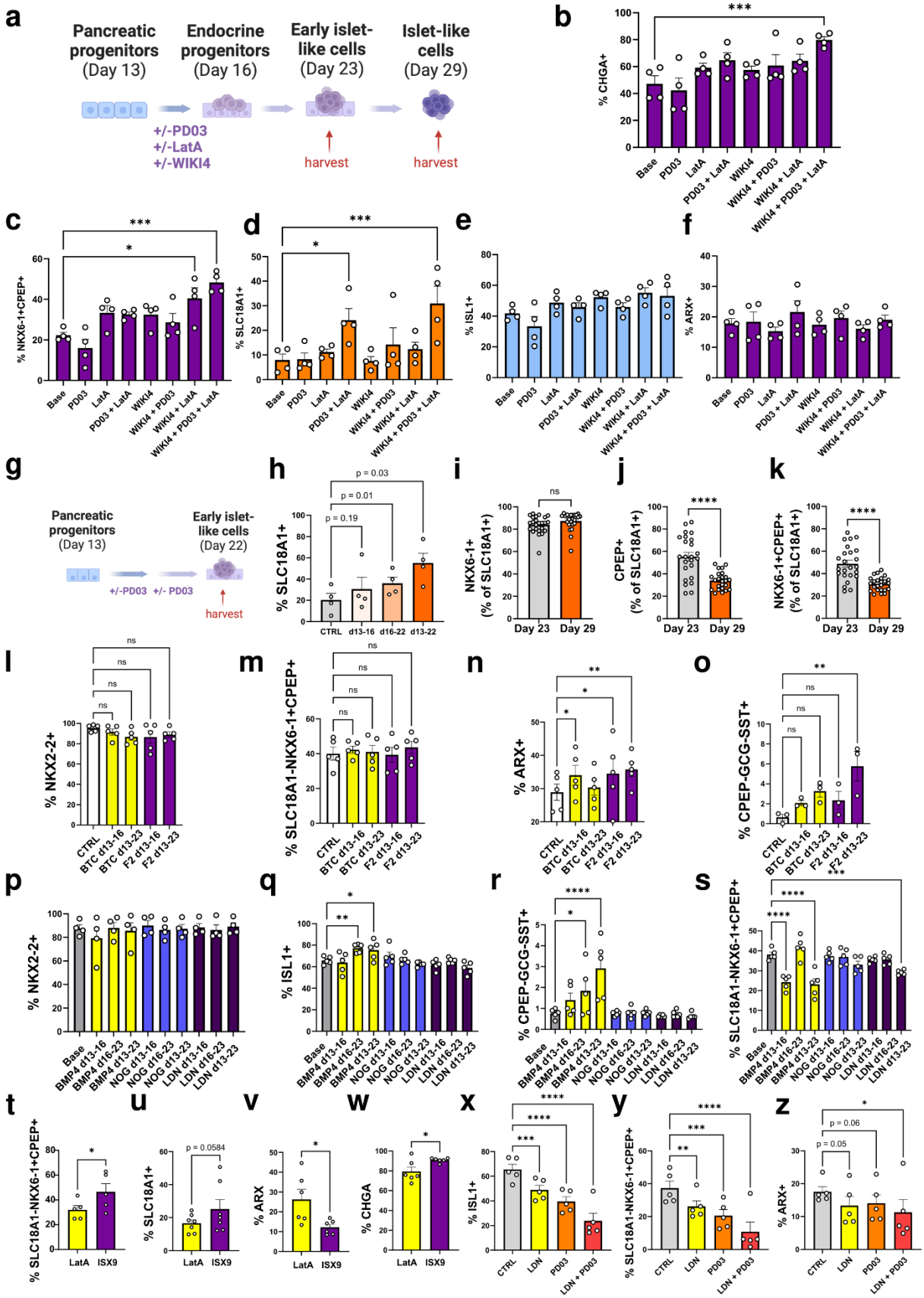
Supplemental Figure 1, related to Figure 1: The effect of Latrunculin A on endodermal and mesodermal differentiation, related to Figure 1: a-c) Quantification of $CXCR4^{+}cKIT^{+}$ cells at day 3 (a, n = 7), $PDX1^{-}NKX6\text{-}1^{-}$ cells at day 13 (b, n = 7), and $CHGA^{+}$ cells at day 23 (c, n = 6) of differentiation as assessed by flow cytometry. Populations compared using one-tailed paired Student's t test. d) Representative flow cytometry plots visualizing day 13 hPSC-PPs generated with and without LatA in the *HES3* hPSC line. e-f) Quantification of $PDX1^{+}NKX6\text{-}1^{+}$ cells (e, n = 11) and $PDX1^{-}NKX6\text{-}1^{-}$ cells (f, n = 11) in day 13 hPSC-PPs generated with and without LatA in the *HES3* hPSC line. Populations compared by one-tailed Wilcoxon test. g) Representative flow cytometry plots visualizing day 13 hPSC-PPs generated with and without LatA in the *ELF1* hPSC line. h-i) Quantification of $PDX1^{+}NKX6\text{-}1^{+}$ cells (h, n = 6) and $PDX1^{-}NKX6\text{-}1^{-}$ cells (i, n = 5) in day 13 hPSC-PPs generated with and without LatA in the *ELF1* hPSC line. Populations compared by one-tailed Wilcoxon test. j) Representative flow cytometry plots visualizing day 3 mesoderm generated with and without LatA. k) Quantification of the effect of LatA on the differentiation of $PDGFRA^{+}$ cells (n = 4) in day 3 mesoderm cells differentiated in control or Y-27631-supplemented base media in the H1 line. Populations compared by ANOVA followed by Holm-Sidak's multiple comparison test. All replicates in this figure are from independent differentiations. Exact p values are available in source data file. Legend: hPSC, human pluripotent stem cell; LatA, Latrunculin A; PP, pancreatic progenitors.

Supplemental Figure 2



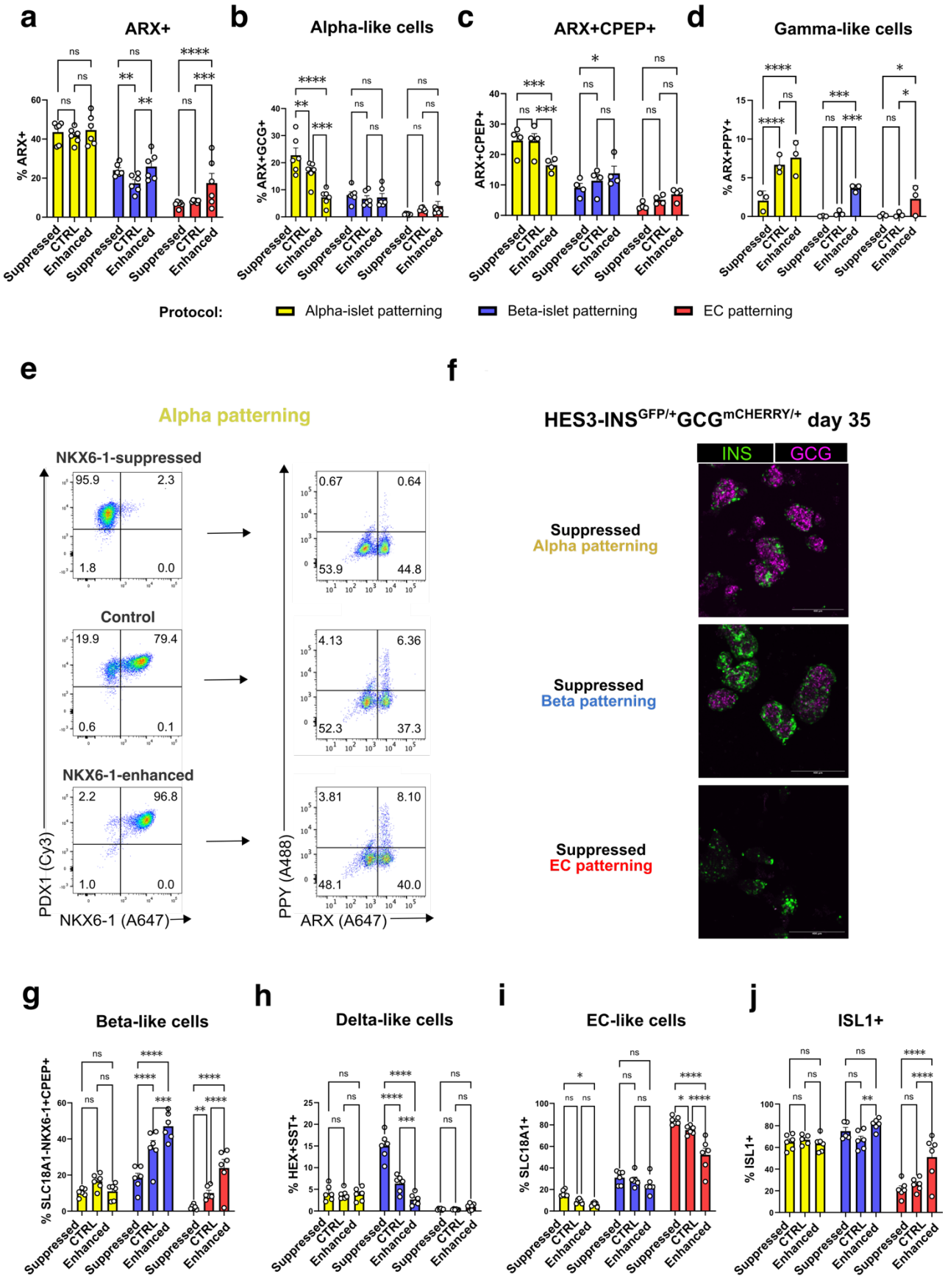
Supplemental Figure 2, related to Figure 2: a-d) Comparison of CTRL and ARW-treated day 13 hPSC-PPs for frequencies of NKX6-1$^+$ (a, n = 4), CDX2$^+$ (b, n = 4), NGN3$^+$ (c, n = 4) and CHGA$^+$ (d, n = 4) cells. e-h) Comparison of CTRL and ARW-treated day 23 hPSC-islets for frequencies of NKX6-1$^+$CPEP$^+$ (e, n = 5), CHGA$^+$ (f, n = 5), GCG$^+$ (g, n = 4) and SLC18A1$^+$ (h, n = 4) cells. For panels a-h, all comparisons done using one-tailed paired Student's t-test. i-l) Comparison of bulk RNA-sequencing expression levels of genes in control and *Isl1*-CKO mouse (time points specified in each panel): *Slc18a1* (i-k), *Tph1* (k), *Tac1* (l). For panels i-l, all comparisons done using one-tailed unpaired Student's t-test. For panels i-h, all replicates in this figure are from independent differentiations. For panels i-l, all replicates are from samples from independent mice. Exact p values are available in source data file. Legend: ARW, Activin A + all-trans retinoic acid + WIKI4; CKO, *Isl1*-CKO; CTRL, control; E, embryonic day; P, post-natal day.

Supplemental Figure 3



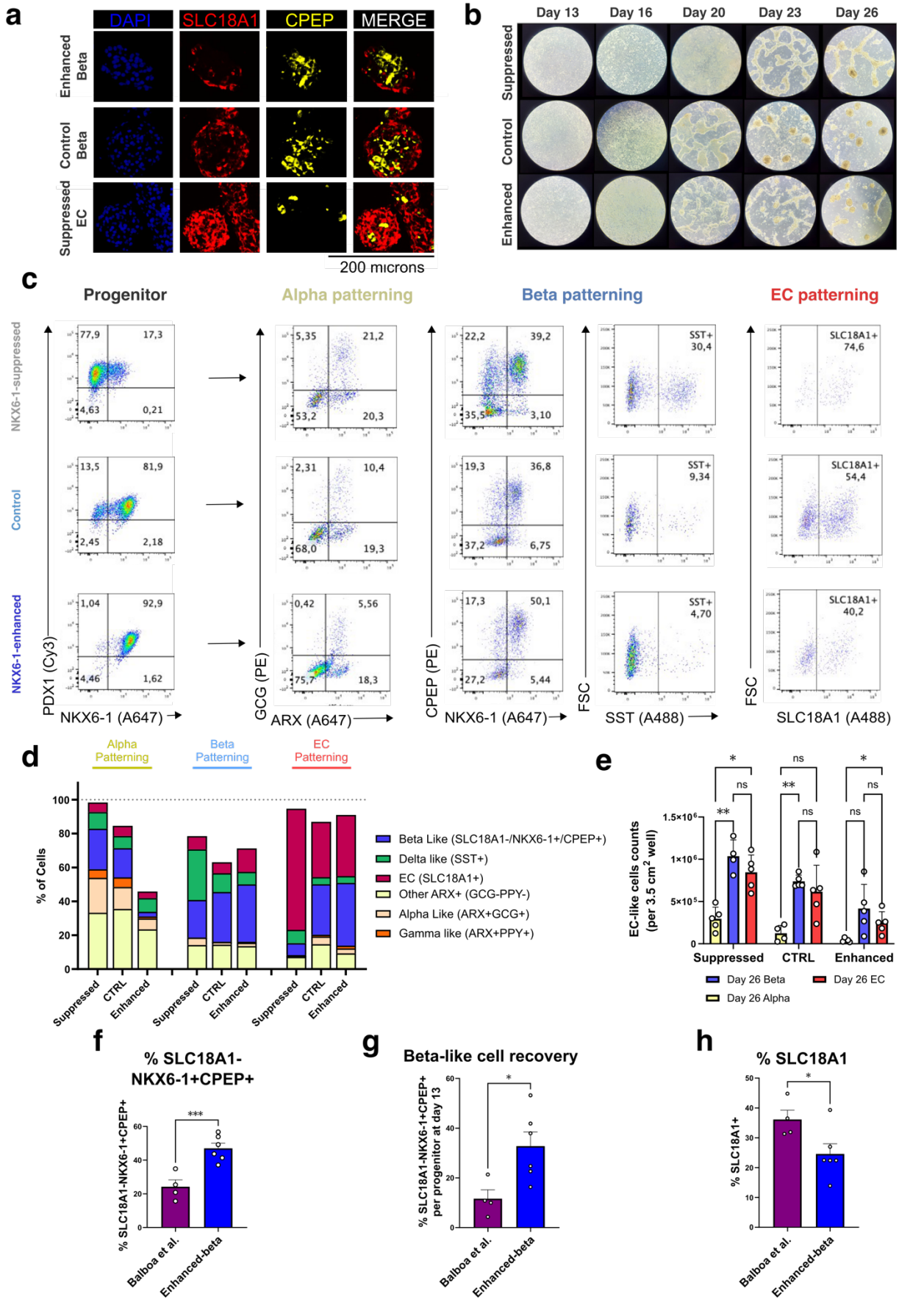
Supplemental Figure 3, related to Figure 3: a) Schematic describing the experimental design for panels b-f. Created in BioRender. Misra, P. (2026) https://BioRender.com/75sa95l. b) Quantification of frequency of $CHGA^+$ (b, n = 4), $NKX6\text{-}1^+CPEP^+$ (c, n = 4), $SLC18A1^+$ (d, n = 4), $ISL1^+$ (e, n = 4), and ARX^+ (f, n = 4) cells as assessed by flow cytometry at day 29 of differentiation across conditions. g) Schematic describing the experimental design for panel h. From days 0-13, factors present in Fig. 1a, including LatA at day 0 were included. From days 13-16, LatA, T3, Y-27632, Heparin and DBZ were retained in addition to the factors tested. From day 16-23, only T3, Y-27632, Heparin and Repsox were retained. Created in BioRender. Misra, P. (2026) https://BioRender.com/f49mbca. h) Quantification of SLC18A1 frequency across conditions studied (n = 4). i-k) Characterization of $SLC18A1^+$ cells at days 23 and 29 for frequencies of $NKX6\text{-}1^+$ (i), $CPEP^+$ (j), and $NKX6\text{-}1^+CPEP^+$ (k) cells, as determined by flow cytometry, across all LatA/WIKI4/PD03 conditions (panels a-f, n = 24). l-o) Quantification of hPSC-islet expression of $NKX2\text{-}2^+$ (l, n = 5), $SLC18A1^-NKX6\text{-}1^+CPEP^+$ (m, n = 5), ARX+ (n, n = 5), and $CPEP^-GCG^-SST^+$ (o, n = 3) cells, as determined by flow cytometry, in response to BTC or F2 (Fig. 3a). p-s) Quantification of hPSC-islet frequencies of $NKX2\text{-}2^+$ (p, n = 4), $ISL1^+$ (q, n = 5), $CPEP^-GCG^-SST^+$ (r, n = 5), and $SLC18A1^-NKX6\text{-}1^+CPEP^+$ cells (s, n = 5), as determined by flow cytometry, in response to either BMP4, LDN or NOG (Fig. 3g). t-w), Quantification of $SLC18A1^-NKX6\text{-}1^+CPEP^+$ (t, n = 5), $SLC18A1^+$ (u, n = 7), ARX^+ (v, n = 6), and $CHGA^+$ (w, n = 6) frequencies as assessed by flow cytometry in d29 cultures treated with either LatA (added for 24 hours on day 13 only) or ISX9 (added for 72 hours from days 13 to 16). x-z) Quantification of hPSC-islet frequencies of $ISL1^+$ (x, n = 5), $SLC18A1^-NKX6\text{-}1^+CPEP^+$ (y, n = 5), and ARX^+ (z, n = 5) cells, as determined by flow cytometry, in response to LDN and/or PD03 (Fig. 3l). For panels b-f, h, l-s, and x-z, comparisons were done using paired one-way ANOVA followed by Holm-Sidak's post-hoc test, comparing each condition against the control. Comparisons not shown did not meet threshold of statistical significance. For panels i-k and t-x, populations were compared by one-tailed paired Student's t-test. For schematics in panels a and g, factors not shown include all factors present in Fig. 1a from day 0-13, including LatA; T3, Y-27632, Heparin, REPSOX, DBZ, FGF10 and ATRA from days 13-23; and T3, Heparin, REPSOX, FGF10 and ATRA from days 13-16. All replicates in this figure are from independent differentiations. Exact p values are available in source data file. Legend: ATRA, all-trans retinoic acid; BTC, betacellulin; CTRL, control; DBZ, dibenzazepine; EC, enterochromaffin; F2, FGF2; ISX9, isoxazole-9; LatA, Latrunculin A; LDN, LDN193189; NOG, NOGGIN; PD03, PD0325901.

Supplemental Figure 4



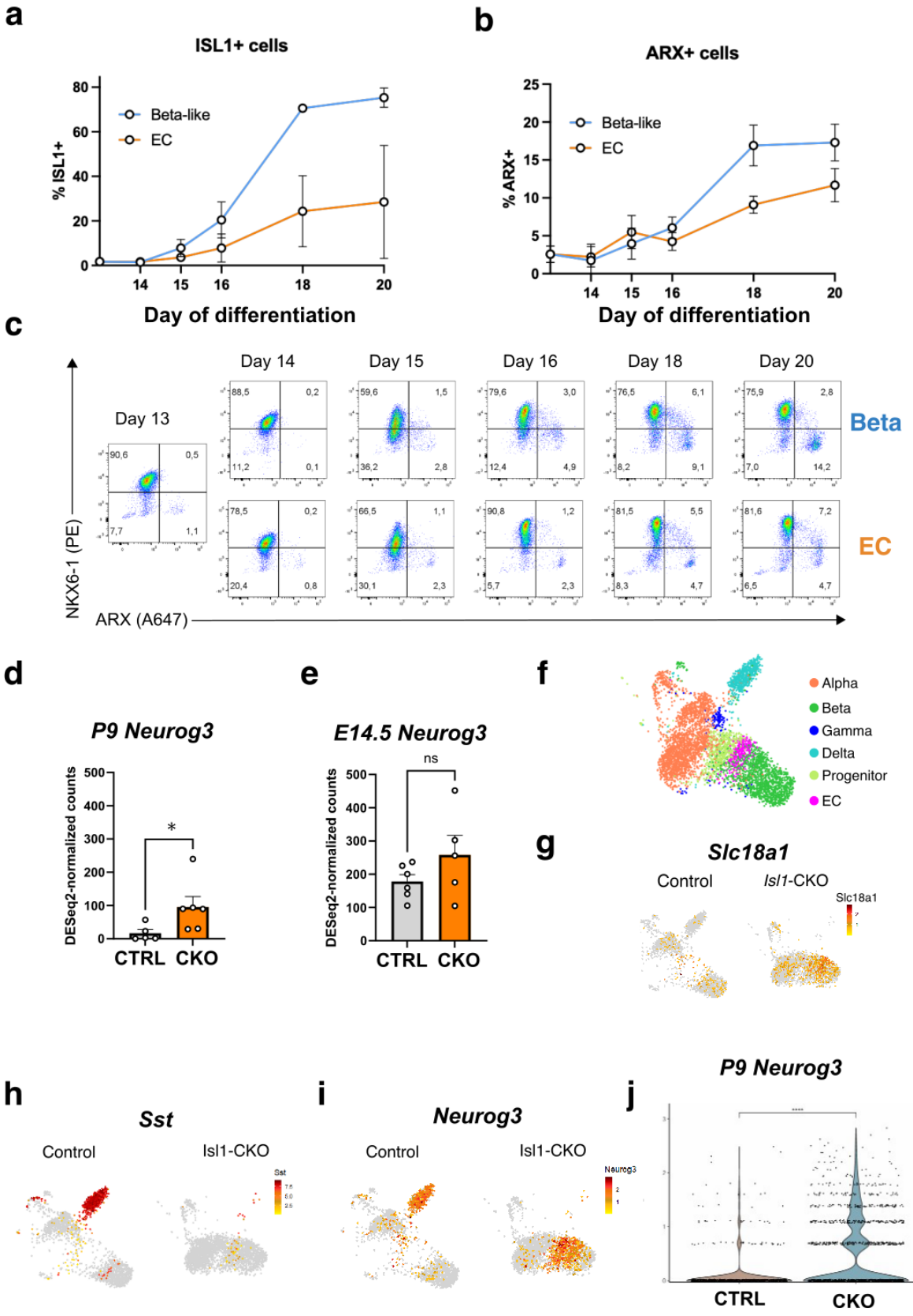
Supplemental Figure 4, related to Figure 4: a-d) Quantification of day 26 hPSC-islet frequencies of ARX^+ (a, n = 6), ARX^+GCG^+ alpha-like (b, n = 6), ARX^+CPEP^+ (c, n = 4), and ARX^+PPY^+ gamma-like (d, n = 3) derived from each of the progenitor-patterning combinations. e) Representative flow cytometry plots for ARX and PPY expression in hPSC-islet cultures derived from NKX6-1-suppressed (top row), control (middle row) and NKX6-1-enhanced (bottom row) hPSC-PPs subjected to alpha patterning. The phenotype of associated hPSC-PPs is shown on the left. f) Representative fluorescence images from n = 3 independent differentiations demonstrating the distribution of *GCG* and *INS* expression in hPSC-islets derived from the HES3 $INS^{GFP/+}GCG^{mCherry//+}$ hESC line differentiated according to the patterning conditions described. Scale bars represent 400 um. g-j) Quantification of day 26 hPSC-islet frequencies of $SLC18A1^-NKX6\text{-}1^+CPEP^+$ beta-like (g, n = 6), $HHEX^+SST^+$ delta-like (h, n = 6), $SLC18A1^+$ EC-like (i, n = 6), and $ISL1^+$ (j, n = 6) cells derived from each of the progenitor-patterning combinations. For panels a-d and g-i, populations were compared by ANOVA followed by Holm-Sidak's post-hoc test. For panel j, populations were compared by Mixed-effects analysis followed by Holm-Sidak's post-hoc test. Error bars denote the mean +/- the SEM. All replicates in this figure are from independent differentiations. Exact p values are available in source data file. Legend: EC, enterochromaffin; hESC, human embryonic stem cells; LatA, Latrunculin A.

Supplemental Figure 5



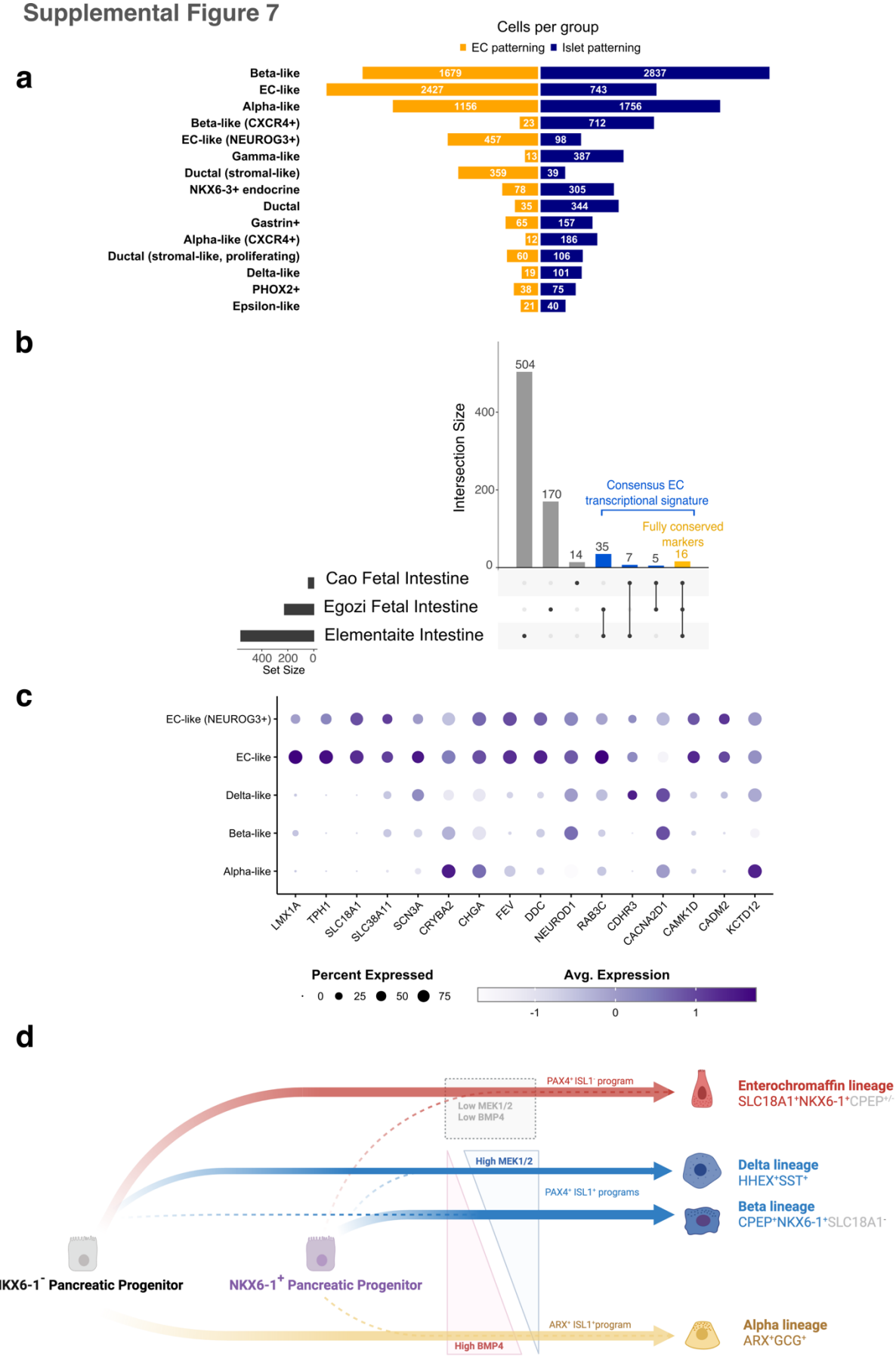
Supplemental Figure 5, related to figure 4: a) Immunofluorescence images for DAPI, SLC18A1, and CPEP in H1 hESCs differentiated via NKX6-1-enhanced progenitors and beta patterning (top), control progenitors and beta patterning (middle), and NKX6-1-suppressed progenitors and EC patterning (bottom). Scale bar represents 200 um. b) Brightfield images of differentiating H1 hESC-derived cultures between day 13 (hPSC-PP stage) and day 26 (hPSC-islet stage) of differentiation, as seen at 10x magnification. Images are representative of n = 2 independent differentiations. c-d) Validation of experimental results for main Fig. 4f-g (H1 hESC line) in the LiPSC-GR1.1 iPSC line. e) Quantification of EC cell yield per well at day 26 for each progenitor-patterning combination tested. f-h) Comparison of H1 hESCs differentiated according to either the protocol by Balboa *et al.*[8] or the "enhanced-beta" protocol described in this work for frequency of beta-like cells (f), yield of beta-like cells per 3.5cm^2 well of hESCs differentiated (g), and frequency of SLC18A1+ EC-like cells (h). For panel e, cell counts at day 13 and day 26 were compared for each type of progenitor using paired one-way ANOVA followed by Holm-Sidak's post-hoc test. For panels f-i, populations were compared using the unpaired one-tailed t-test. In all cases, error bars denote the mean +/- SEM. All replicates in this figure are from independent differentiations. Exact p values are available in source data file. Legend: EC, enterochromaffin; hESC, human embryonic stem cells; iPSC, induced pluripotent stem cells.

Supplemental Figure 6



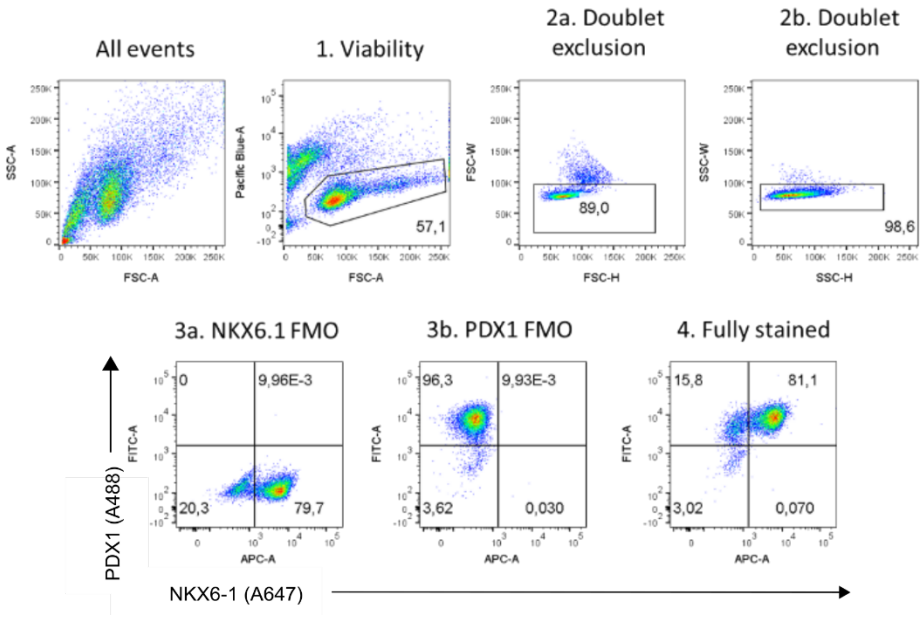
Supplemental Figure 6, related to Figure 5: a-b) Quantification of the frequencies of $ISL1^+$ (a) and ARX^+ (b) cells following beta cell or EC differentiation (n = 3 independent differentiations each). Error bars represent median +/- SD. c) Representative flow cytometric plots characterizing ARX and NKX6-1 expression of NKX6-1-enhanced hPSC-PPs undergoing either beta-islet or EC patterning. d-e) Comparison of bulk RNA-sequencing expression levels of *Neurog3* in control and *Isl1*-CKO mouse at E9 (d) and P14.5 (e). Comparisons done using one-tailed unpaired Student's t-test with Welch's correction. Error bars denote the mean +/- SEM. Replicates are from samples taken from independent mice. f) UMAP demonstrating endocrine clusters in sorted mouse $tdTomato^+$ endocrine cells from single cell RNA-sequencing. g-j) Split feature plots highlighting *Slc18a1* (g), *Sst* (h), and *Neurog3* (i) expression in Control and *Isl1*-CKO endocrine cells. (j) Single cell RNA-seq violin plots displaying the distribution of *Neurog3* transcript levels across individual cells at postnatal day P9. Wilcoxon test, p = 1.4e-65. Exact p values for other panels are available in source data file. Legend: EC, enterochromaffin; E, embryonic day; P, post-natal day.

Supplemental Figure 7



Supplemental Figure 7, related to Figure 6: a) Mirror plot demonstrating the number of cells across clusters and patterning conditions. b) UpSet plot demonstrating the degree of overlap between marker genes from 3 distinct single cell RNA sequencing datasets of human intestinal EC cells. c) Dot plot depicting expression patterns of 16 fully conserved EC markers across the major hPSC-islet endocrine clusters. d) Proposed model for endocrine lineage allocation of hPSC-PPs, as suggested by the data generated in this study. Created in BioRender. Misra, P. (2026) https://BioRender.com/dulp5ud
Legend: Avg, average; EC, enterochromaffin; hPSC, human pluripotent stem cells; PPs, pancreatic progenitors.

Supplemental Figure 8



Supplemental Figure 8: Gating strategy used for all flow cytometry analyses, as shown for detection of PDX1 and NKX6-1 expression at day 13 of differentiation. Legend: FMO, fluorescence minus one.

Supplemental Table 1: EC marker genes - overlap of 3 intestinal datasets

LMX1A
TPH1
SLC18A1
SLC38A11
SCN3A
CRYBA2
CHGA
FEV
DDS
NEUROD1
RAB3C
CDHR3
CACNA2D1
CAMK1D
CADM2
KCTD12

Supplemental Table 2: Differentiation Protocol

Media used in stage 1 (day 0)

hPSC differentiation: On day 0, confluent cultures of hPSCs in monolayer format were washed with Calcium- and Magnesium-free dPBS (Corning #20-031-CV) and treated with components of stage 1 media. The media used for each stage are specified in the tables below (Tables 2.1-2.8), with alterations specified in the text where relevant. When reagents were obtained from suppliers as liquids, the concentration is provided as a percentage of reagent volume/ base media volume. When reagents were provided as desiccated powders that required reconstitution, the concentration is specified in either mass or molarity per unit volume of fully reconstituted media. All media were supplemented with 1% vol/vol Gibco Penicillin-Streptomycin (ThermoFisher Scientific #15070063).

Reagent	Concentration	Supplier
RPMI	Base media	Wisent
Gibco Bicarbonate	2% (v/v)	ThermoFisher Scientific
Gibco Non-essential amino acids	1% (v/v)	ThermoFisher Scientific
B27^{TM} supplement, minus insulin	1% (v/v)	ThermoFisher Scientific
Hyclone Glutamine	1% (v/v)	Fisher scientific
Recombinant human Activin A	100 ng/mL	Bio-techne
CHIR99021	3 µM	Tocris Biosciences
Y-27652	10 µM	Sigma
Latrunculin A*	0.24-0.4% (v/v)	Cayman Chemical

Legend: v/v (volume/volume); *Used in select experiments as indicated in text and figures.

Balboa et al. (stage 1)

For differentiation according to Balboa et al., undifferentiated cells were replated at a density of 180,000 - 200,000 cells/cm2. Differentiation was started 24 hours later.

Reagent	Concentration	Supplier
MCDB131	Base media	Wisent
Hyclone Glutamine	1% (v/v)	Fisher scientific
Gibco Bicarbonate	2% (v/v)	ThermoFisher Scientific
Proliant Bovine Serum Albumin (BSA), fatty acid-free	5 g/L	Fisher Scientific
D-Glucose	5 mM added to base media	Millipore Sigma
Ascorbic Acid	50mg/mL	Sigma
Recombinant human Activin A	100 ng/mL	Bio-techne
CHIR99021	3 µM	Tocris Biosciences
Y-27652	10 µM	Sigma

Legend: v/v (volume/volume)

Media used in stage 2 (days 1-2, media changed daily)

Reagent	Concentration	Supplier
RPMI	Base media	Wisent
Gibco Bicarbonate	2% (v/v)	ThermoFisher Scientific
Gibco Non-essential amino acids	1% (v/v)	ThermoFisher Scientific
Hyclone Glutamine	1% (v/v)	Fisher scientific
B27^{TM} supplement, minus insulin	1% (v/v)	ThermoFisher Scientific
Recombinant human Activin A	100 ng/mL	Bio-techne

Legend: v/v (volume/volume)

Balboa et al. (stage 2)

Reagent	Concentration	Supplier
MCDB131	Base media	Wisent
Hyclone Glutamine	1% (v/v)	Fisher scientific
Gibco Bicarbonate	2% (v/v)	ThermoFisher Scientific
Proliant Bovine Serum Albumin (BSA), fatty acid-free	5 g/L	Fisher Scientific
D-Glucose	5 mM added to base media	Millipore Sigma
Ascorbic Acid	50 ug/mL	Sigma
Recombinant human Activin A	100 ng/mL	Bio-techne
CHIR99021 (added only on day 1 and not on day 2)	0.3 μM	Tocris Biosciences

Legend: v/v (volume/volume)

Media used in stage 3 (days 3-5, media changed daily)

Reagent	Concentration	Supplier
MCDB131	Base media	Wisent
Proliant Bovine Serum Albumin (BSA), fatty acid-free	5 g/L	Fisher Scientific
Hyclone Glutamine	1% (v/v)	Fisher scientific
MACS NeuroBrew-21	1% (v/v)	Miltenyi Biotec
FGF10	50 ng/mL	Bio-techne
Dorsomorphin	0.75 µM	Millipore Sigma
Recombinant human Activin A*	7.5-10 ng/mL	Bio-techne
All-trans Retinoic Acid*	0.5-2.5 µM	Millipore Sigma
WIKI4*	0.25 µM	Tocris Biosciences

Legend: v/v (volume/volume) ; *Used in select experiments as indicated in text and figures.

Balboa et al. (stage 3)

Note: Prior to adding day 3 media, wash cells with sterile dPBS (without calcium and magnesium). Be gentle, as cells may sometimes peel. Cells are fed daily.

Reagent	Concentration	Supplier
MCDB131	Base media	Wisent
Hyclone Glutamine	1% (v/v)	Fisher scientific
Gibco Bicarbonate	2% (v/v)	ThermoFisher Scientific
Proliant Bovine Serum Albumin (BSA), fatty acid-free	5 g/L	Fisher Scientific
D-Glucose	5 mM added to base media	Millipore Sigma
Ascorbic Acid	50 ug/mL	Sigma
FGF7/KGF	50 ng/mL	Bio-techne

Legend: v/v (volume/volume)

Media used in stage 4 (days 6-7, media changed daily)

Reagent	Concentration	Supplier
Gibco DMEM-HG	Base media	ThermoFisher Scientific
Hyclone Glutamine	1% (v/v)	Fisher scientific
MACS NeuroBrew-21	1% (v/v)	Miltenyi Biotec
Ascorbic acid	50 µg/mL	Millipore Sigma
FGF10	50 ng/mL	Bio-techne
Recombinant human Noggin Fc Chimera	50 ng/mL	Bio-techne
Sant-1	0.25 µM	Tocris Biosciences
All-trans Retinoic Acid	2 µM	Millipore Sigma

Legend: v/v (volume/volume)

Balboa et al. (stage 4)

Reagent	Concentration	Supplier
MCDB131	Base media	Wisent
Hyclone Glutamine	1% (v/v)	Fisher scientific
Gibco Bicarbonate	2% (v/v)	ThermoFisher Scientific
Proliant Bovine Serum Albumin (BSA), fatty acid-free	20 g/L	Fisher Scientific
D-Glucose	5 mM added to base media	Millipore Sigma
Ascorbic Acid	50 ug/mL	Sigma
ITS-X	0.5% (v/v)	Gibco
Sant-1	0.25 µM	Tocris Biosciences
LDN193189	100 nM	Cayman Chemical
TPPB	200 nM	Tocris Biosciences
FGF7/KGF	50 ng/mL	Bio-techne
All-trans Retinoic Acid	1 µM	Millipore Sigma

Legend: v/v (volume/volume)

Media used in stage 5 (days 8-12, media changed on days 8, 10 and 12)

Reagent	Concentration	Supplier
Gibco DMEM-HG	Base media	ThermoFisher Scientific
Hyclone Glutamine	1% (v/v)	Fisher scientific
MACS NeuroBrew-21	1% (v/v)	Miltenyi Biotec
Ascorbic acid	50 µg/mL	Millipore Sigma
FGF10	50 ng/mL	Bio-techne
Recombinant human Noggin Fc Chimera	50 ng/mL	Bio-techne
Nicotinamide	10 mM	Millipore Sigma
Recombinant human EGF protein	100 ng/mL	Bio-techne
Sodium Butyrate	1 mM	Cayman chemical
All-trans Retinoic Acid*	2 µM	Millipore Sigma
SB431542*	10 µM	Millipore Sigma

Legend: v/v (volume/volume) ; *Used in select experiments as indicated in text and figures.

Balboa et al. (stage 5)

Note that the duration of this stage is 4 days (days 8-11), and thus stage 6 begins on day 12. On day 11, cells are fed by adding 2 mL of stage 4 pre-warmed media per well.

Reagent	Concentration	Supplier
MCDB131	Base media	Wisent
Hyclone Glutamine	1% (v/v)	Fisher scientific
Gibco Bicarbonate	2% (v/v)	ThermoFisher Scientific
Proliant Bovine Serum Albumin (BSA), fatty acid-free	20 g/L	Fisher Scientific
D-Glucose	5 mM added to base media	Millipore Sigma
Ascorbic Acid	50 ug/mL	Sigma
ITS-X	0.5% (v/v)	Gibco
Sant-1	0.25 µM	Tocris Biosciences
LDN193189	100 nM	Cayman Chemical
TPPB	100 nM	Tocris Biosciences
FGF7/KGF	2 ng/mL	Bio-techne
All-trans Retinoic Acid	0.1 µM	Millipore Sigma
Nicotinamide	10 mM	Millipore Sigma
Recombinant human Activin A	10 ng/mL	Bio-techne
Y-27652	10 µM	Tocris Biosciences
Recombinant human EGF protein	100 ng/mL	Bio-techne

Legend: v/v (volume/volume)

Media used in stage 6 (days 13-15, media changed only on day 13)

On day 13, some experiments were aggregated by single cell dissociation using TrypLE (Life Technologies #12605) with 1% Bovine Pancreas DNase I (Millipore Sigma #260913) 1 mg/mL, centrifuged for 5 min at 1000 RPM, and resuspended in base medium at 2 million cells/mL supplemented with 1% DNase 1 mg/mL (Calbiochem 260913).

Reagent	Concentration	Supplier
MCDB131	Base media	Wisent
Proliant BSA, fatty acid-free	10 g/L	Fisher Scientific
3,3′,5-Triiodo-L-thyronine	1 µM	Millipore Sigma
Gibco Bicarbonate	2% (v/v)	ThermoFisher Scientific
Hyclone Glutamine	1% (v/v)	Fisher scientific
MACS NeuroBrew-21	1% (v/v)	Miltenyi Biotec
D-Glucose	15 mM (added to base media)	Millipore Sigma
FGF10	50 ng/mL	Bio-techne
Heparin	10 µg/mL	Millipore Sigma
All-trans Retinoic Acid	0.1 µM	Millipore Sigma
Zinc Sulfate	10 µM	Millipore Sigma
REPSOX (Alk 5 inhibitor II)	10 µM	Tocris Biosciences
Dibenzazipine	1 µM	Tocris Biosciences
Y-27652	10 µM	Tocris Biosciences
LDN193189*	100 nM	Cayman Chemical
PD0325901*	10 µM	Tocris Biosciences
WIKI4*	1 µM	Tocris Biosciences
FGF2*	10 ng/mL	Bio-techne
Betacellulin*	100 ng/mL	Bio-techne
Recombinant human BMP4*	10 ng/mL	Bio-techne
Recombinant human Noggin Fc Chimera*	50 ng/mL	Bio-techne
Latrunculin A*§	0.5 µM	Tocris Biosciences
Isoxazole-9*	10 µg/mL	EMD Millipore

Legend: v/v (volume/volume); *Used in select experiments as indicated in text and figures.
[§]In some experiments where Latrunculin A was added to stage 6 media, media was changed on day 14 to stage 6 media without Latrunculin A.

Balboa et al. (stage 6)

Note that the duration of this stage is 4 days, and cells are fed daily. On day 12 and 13, exchange 4 mL of spent media with 4 mL fresh stage 6 media per well. On day 14, transfer aggregates from AggreWell plate to small 60 mm petri dishes (Falcon) for suspension culture using 5 mL of pre-warmed stage 6 media per dish. On day 16, perform a full media change with 5 mL pre-warmed stage 6 media.

Reagent	Concentration	Supplier
MCDB131	Base media	Wisent
Proliant Bovine Serum Albumin (BSA), fatty acid-free	20 g/L	Fisher Scientific
GC1	1 μM	Tocris Biosciences
Gibco Bicarbonate	2% (v/v)	ThermoFisher Scientific
Hyclone Glutamine	1% (v/v)	Fisher scientific
ITS-X	0.5% (v/v)	Gibco
D-Glucose	15 mM (added to base media)	Millipore Sigma
Sant-1	0.25 μM	Tocris Biosciences
Heparin	10 μg/mL	Millipore Sigma
All-trans Retinoic Acid	0.05 μM	Millipore Sigma
Zinc Sulfate	10 μM	Millipore Sigma
LDN193189	100 nM	Cayman Chemical
REPSOX (Alk 5 inhibitor II)	10 μM	Tocris Biosciences
Dibenzazipine	0.1 μM	Tocris Biosciences
Y-27652	10 μM	Tocris Biosciences
Betacellulin	20 ng/mL	Bio-techne

Legend: v/v (volume/volume)

Media used in stage 7 (day 16 until either day 20 or 23)

The duration of this stage is between 4-7 days (see text). Media is changed on days 16, and if extending until day 23, then media is changed also on day 23

Reagent	Concentration	Supplier
MCDB131	Base media	Wisent
BSA, fatty acid-free	10 g/L	Fisher Scientific
3,3′,5-Triiodo-L-thyronine	1 µM	Millipore Sigma
Gibco Bicarbonate	2% (v/v)	ThermoFisher Scientific
Hyclone Glutamine	1% (v/v)	Fisher scientific
MACS NeuroBrew-21	1% (v/v)	Miltenyi Biotec
Ascorbic acid	50 µg/mL	Millipore Sigma
D-Glucose	15 mM (added to base media)	Millipore Sigma
FGF10	50 ng/mL	Bio-techne
Heparin	10 µg/mL	Millipore Sigma
All-trans Retinoic Acid	0.1 µM	Millipore Sigma
Zinc Sulfate	10 µM	Millipore Sigma
REPSOX	10 µM	Tocris Biosciences
Dibenzazipine	1 µM	Tocris Biosciences
LDN193189*	100 nM	Cayman Chemical
WIKI4*	1 µM	Tocris Biosciences
PD0325901*	10 µM	Tocris Biosciences
FGF2*	10 ng/mL	Bio-techne
Betacellulin*	100 ng/mL	Bio-techne
Recombinant human BMP4*	10 ng/mL	Bio-techne
Recombinant human Noggin Fc Chimera*	50 ng/mL	Bio-techne

Legend: v/v (volume/volume); *Used in select experiments as indicated in text and figures.

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The duration of this stage is 8 days. Cells are fed on day 16 and subsequently media changes are performed three times a week (every 2-3 days).

Reagent	Concentration	Supplier
MCDB131	Base media	Wisent
Proliant Bovine Serum Albumin (BSA), fatty acid-free	20 g/L	Fisher Scientific
GC1	1 µM	Tocris Biosciences
Gibco Bicarbonate	2% (v/v)	ThermoFisher Scientific
Hyclone Glutamine	1% (v/v)	Fisher scientific
ITS-X	0.5% (v/v)	Gibco
D-Glucose	15 mM (supplemented added to base media)	Millipore Sigma
Heparin	10 µg/mL	Millipore Sigma
Zinc Sulfate	10 µM	Millipore Sigma
LDN193189	100 nM	Cayman Chemical
REPSOX (Alk 5 inhibitor II)	10 µM	Tocris Biosciences
Dibenzazipine	0.1 µM	Tocris Biosciences

Legend: v/v (volume/volume)

Media used in stage 8 (Starting day 20 or 23, media changed every 3 days)

Reagent	Concentration	Supplier
MCDB131	Base media	Wisent
BSA, fatty acid-free	5 g/L	Fisher Scientific
Gibco Bicarbonate	2% (v/v)	ThermoFisher Scientific
Hyclone Glutamine	1% (v/v)	Fisher scientific
Gibco Non-essential amino acids	1% (v/v)	ThermoFisher Scientific
D-Glucose	1.5 mM (added to base media)	Millipore Sigma
Zinc Sulfate	10 µM	Millipore Sigma
ZM 447439*	1 µM	SelleckChem
N-Acetyl Cysteine*	1 mM	Millipore Sigma

Legend: v/v (volume/volume); *Used in select experiments as indicated in text and figures.

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Reagent	Concentration	Supplier
CMRL-1066	Base media	Corning
Hyclone Glutamine	1% (v/v)	Fisher scientific
Sodium pyruvate	0.5 mM	Gibco
Heparin	10 µg/mL	Millipore Sigma
Zinc Sulfate	10 µM	Millipore Sigma
ZM 447439	0.5 µM	SelleckChem
N-Acetyl Cysteine	1 mM	Millipore Sigma
3,3′,5-Triiodo-L-thyronine	10 nM	Millipore Sigma
Lipid concentrate	0.5x (0.05% v/v)	Invitrogen
Trace-element A	1x (1% v/v)	Corning
Trace-element B	1x (1% v/v)	Corning

Supplemental Table 3: Materials

Compounds

Compound Name	Company	Catalogue number
IMDM	Life Technologies	12440
StemMACS iPS-brew XF	Miltenyi Biotec	130-104-368
Fetal Bovine Serum (FBS)	Gibco	12483-020
DMSO	Millipore Sigma	D2650
DMEM:F12	Wisent	319-085-CL
Y-27632	Tocris Biosciences	1254
TrypLE	Life Technologies	12605
DNase I	Calbiochem	260913
RPMI	Wisent	350-000-CL
Gibco Bicarbonate	ThermoFisher Scientific	25080094
Gibco Non-essential amino acids	ThermoFisher Scientific	11140050
B27 supplement, minus insulin	ThermoFisher Scientific	A1895601
Hyclone Glutamine	Fisher Scientific	SH30034.01
Recombinant human Activin A	Bio-techne	338-AC
CHIR99021	Tocris Biosciences	1254
Latrunculin A	Cayman Chemical	10010630
MCDB131	Wisent	301-015-CL
Proliant Bovine Serum Albumin (BSA)	Fisher Scientific	68700
MACS NeuroBrew-21	Miltenyi Biotec	130-097-263
FGF10	Bio-techne	345-FG
Dorsomorphin	Millipore Sigma	P5499
DMEM-HG	ThermoFisher Scientific	11965
All-trans Retinoic Acid	Millipore Sigma	R2625
WIKI4	Tocris Biosciences	4855
Ascorbic acid	Millipore Sigma	A4544
Recombinant human Noggin Fc Chimera	Bio-techne	3344-NG
Sant-1	Tocris Biosciences	1974
Nicotinamide	Millipore Sigma	N0636
Recombinant human EGF protein	Bio-techne	236-EG
Sodium Butyrate	Cayman Chemical	13121

3,3′,5-Triiodo-L-thyronine	Millipore Sigma	T6397
D-Glucose	Millipore Sigma	G7021
Heparin	Millipore Sigma	H3149
Zinc Sulfate	Millipore Sigma	Z0251
LDN193189	Cayman Chemical	11802
REPSOX	Tocris Biosciences	3742
Dibenzazipine	Tocris Biosciences	4489
PD0325901	Tocris Biosciences	4192
FGF2	Bio-techne	233-FB
Betacellulin	Bio-techne	261-CE-050
Recombinant human BMP4	Bio-techne	314-BP
Isoxazole-9	EMD Millipore	480743
ZM 447439	SelleckChem	S1103
N-Acetyl Cysteine	Millipore Sigma	A9165
Trace-Elements A	Corning	25-021-CI
Trace-Elements B	Corning	25-022-CI
Lipid concentrate	Gibco	11905031
ITS-X	Gibco	51500056
FGF7/ KGF	Tocris Biosciences	251-KG
TPPB	Tocris Biosciences	5343
GC1	Tocris Biosciences	4554
CMRL1066	Gibco	15-110-CV

Primary Antibodies

Antibody	**Dilution (FC)**	**Dilution (IF)**	**Vendor**	**Catalogue number**
Mouse anti-cKIT, PE	1:200		Life Technologies	CD11704
Mouse anti-CXCR4, APC	1:200		BD	555976
Mouse anti-ARX, UC	1:2000		Millipore Sigma	MABN102
Goat anti-Brachyury, UC	1:500		R&D Systems	AF2085
Rat anti-C-peptide, UC	1:40	1:50	DSHB	GN-ID4
Rabbit anti-CDX2, UC	1:2000		Abcam	ab76541
Rabbit anti-CHGA, UC	1:1000		Abcam	ab15160
Rabbit anti-FOXA2, UC	1:2000		Abcam	Ab40874
Goat anti-Ghrelin, UC	1:1000		Santa Cruz	sc-10368
Mouse anti-Glucagon, UC	1:2000	1:1000	Sigma	G2654
Mouse anti-Glucagon, PE	1:100		BD	565860
Rabbit anti-HHEX, UC	1:200		Biotechne	MAB83771
Guinea pig anti-Insulin, UC		1:50	Genetex	GTX27842

Mouse anti-ISL1/2, UC	1:2000		BD	Q11-465
Sheep anti-NGN3, UC	1:200		R&D Systems	AF3444
Rabbit anti-NKX2.1, UC	1:500	1:300	Abcam	Ab76013
Mouse anti-NKX2.2, UC	1:200 (Final: 1mg /mL)	1:100	DHSB	74.5A5
Mouse anti-NKX6.1, UC	1:1000		DSHB	F55A10
Mouse anti-NKX6.1, PE	1:1000		BD	563023
Mouse anti-PAX6, PE	1:100		BD	561552
Goat PDX1, UC	1:100		R&D Systems	AF2419
Rabbit anti-PPY, UC	1:6000		Peninsula Laboratorie	T4088
Rabbit anti-SLC18A1, UC	1:300	1:1000	Sigma	HPA063797
Mouse anti-SST, Alexa488	1:200		BD	566032
Rat anti-Serotonin, UC		1:1000	Novus	NB100-65037SS
Rabbit anti-SOX2, UC	1:300		Cell Signaling Technologies	3579
Rabbit anti-SOX9, UC	1:8000		Millipore Sigma	AB5535
Goat anti-SOX17, UC	1:2000		Biotechne	AF1924
Mouse anti-KDR, PE	5:100		R&D Systems	FAB357P
Mouse anti-PDGFRa, APC	5:100		R&D Systems	FAB1264A

UC = unconjugated

Secondary Antibodies

Antibody	**Dilution (FC)**	**Dilution (IF)**	**Vendor**	**Catalogue number**
Donkey anti-Mouse, Alexa647	1:400	1:800	Invitrogen	A31571
Donkey anti-Mouse, Alexa594	1:400		Invitrogen	SA5-10168
Donkey anti-Rabbit, Alexa488	1:400	1:800	Jackson Immuno Labs	711-545-152

Donkey anti-Rabbit, Alexa647		1:500	Jackson Immuno Labs	711-605-152
Donkey anti-Rabbit, Alexa647	1:2000	1:800	Invitrogen	A31573
Donkey anti-Rabbit, Alexa555		1:800	Invitrogen	A31572
Donkey anti-Goat, Cy3	1:400		Jackson Immuno Labs	705-165-167
Donkey anti-Goat, Alexa488	1:400		Jackson Immuno Labs	705-546-147
Donkey anti-Goat, DyLight405		1:500	Jackson Immuno Labs	705-475-147
Donkey anti-Guinea pig, Alexa647		1:500	Jackson Immuno Labs	706-605-148
Donkey anti-Rat, Alexa650	1:400		Invitrogen	SA5-10029
Donkey anti-Rat, Alexa488		1:500	Jackson Immuno Labs	712-547-003
Goat anti-Rat, PE	1:400		BD	550767
Donkey anti-Rat, DyLight488	1:400		Invitrogen	SA5-10026
Donkey anti-Sheep, Alexa594	1:2000		Invitrogen	A-11016

Supplemental Table 4: Genotyping primers

Gene	Sequence
Cre_F	CCATTTTGCAGTGGACTCCT
Cre_R	ACGGACAGAAGCATTTTCCA
Isl1_F	CTCTGGAACATCCCACATTG
Isl1_R	GATGCAACCCCTGTTCCTAC
tdTomato F	CTGTTCCTGTACGGCATGG
tdTomato R	GGCATTAAAGCAGCGTATCC