

Maintenance and SC-islet differentiation of H1 hESCs

All experiments with hESCs were approved by the University of California, San Diego (UCSD), Institutional Review Board and Embryonic Stem Cell Research Oversight Committee (protocol 090165ZX). H1 hESCs were maintained as described²⁹. In brief, hESCs were seeded onto Matrigel (Corning, #356238) coated tissue culture surfaces in mTeSR1 media (Stem Cell Technologies, #85850) supplemented with 1% Penicillin-Streptomycin (Thermo Fisher Scientific, #15140122), and propagated every 3 to 4 days. Accutase (Thermo Fisher Scientific, #00-4555-56) based enzymatic dissociation method was employed for passaging and 10 μ M Y-27632 (Stem Cell Technologies, #72307) was supplied on the first day of each passage.

H1 hESCs were differentiated into SC-islets with a protocol we modified from previous publications^{14,30}. After dissociation using Accutase, 5×10^6 cells were seeded in 2 mL mTeSR1 supplemented with Y-27632 (Stem Cell Technologies) in Matrigel-coated 6-well plate in a 37°C incubator. The following day (day 0), undifferentiated cells were washed in Stage 1/2 base medium (see below) and then differentiated using a seven-step protocol with stage-specific medium. Medium was refreshed daily until day 32 and every two days thereafter. On day 29, cells were dissociated with Accutase, suspended in Stage 7 medium (see below) supplemented with 10 μ M Y-27632 and re-aggregated at a concentration of 3×10^6 cells/well in a low attachment 6-well plate on an orbital shaker (100 rpm) in a 37 °C incubator. The speed of the shaker was increased to 118 rpm on the following day.

All stage-specific base media were comprised of MCDB 131 medium (Thermo Fisher Scientific, #10372-019) supplemented with NaHCO_3 (Sigma-Aldrich, #S6297),

GlutaMAX (Thermo Fisher Scientific, #35050061), D-Glucose (Thermo Fisher Scientific, #D161), and BSA (Life Technologies, #15260-037) using the following concentrations: Stage 1/2 base medium: MCDB 131 medium, 1.5 g/L NaHCO₃, 1X GlutaMAX, 10 mM D-Glucose, 0.5% BSA. Stage 3/4 base medium: MCDB 131 medium, 2.5 g/L NaHCO₃, 1X GlutaMAX, 10 mM D-glucose, 2% BSA, 1% P/S (Thermo Fisher Scientific, #15140122). Stage 5/6 medium: MCDB 131 medium, 1.5 g/L NaHCO₃, 1X GlutaMAX, 20 mM D-glucose, 2% BSA, 1% P/S. Stage 7 medium: MCDB 131 medium, 1.5 g/L NaHCO₃, 1X GlutaMAX, 5.5 mM D-glucose, 2% BSA, 1% P/S.

Media compositions for each stage were as follows: Stage 1 (days 0–3): base medium, 100 ng/mL Activin A (R&D, #338-AC/CF), 25 ng/mL Wnt3a (day 0; R&D, #1324-WN/CF). Day 1–3: base medium, 100 ng/mL Activin A. Stage 2 (days 4–6): base medium, 0.25 mM l-Ascorbic Acid (Vitamin C; Sigma-Aldrich, #A4544), 50 ng/mL FGF (R&D, #251-KG/CF). Stage 3 (days 7–8): base medium, 0.25 mM l-Ascorbic Acid, 50 ng/mL FGF7, 0.25 µM SANT-1 (Sigma-Aldrich, #S4572), 1 µM Retinoic Acid (Sigma-Aldrich, #R2625), 100 nM LDN193189 (Stemgent, #04-0074), 1:200 ITS-X (Thermo Fisher Scientific, #51500056), 200 nM TPB (Calbiochem, #565740). Stage 4 (days 9–11): base medium, 0.25 mM l-Ascorbic Acid, 2 ng/mL FGF7, 0.25 µM SANT-1, 0.1 µM Retinoic Acid, 200 nM LDN193189, 1:200 ITS-X, 100 nM TPB. Stage 5 (days 12–14): base medium, 0.25 µM SANT-1, 0.05 µM RA, 100 nM LDN-193189, 1 µM T3 (Sigma-Aldrich, #T6397), 10 µM ALK5i II (Enzo Life Sciences, #ALX-270-445; Cayman Chemicals, #14794), 10 µM ZnSO₄ (Sigma-Aldrich, #Z0251), 10 µg/mL heparin (Sigma-Aldrich, #H3149), 1:200 ITS-X. Stage 6 (days 15–21): base medium, 100 nM GSiXX, 100 nM LDN-193189, 1 µM T3, 10 µM ALK5i II, 10 µM ZnSO₄, 10 µg/mL heparin, 1:200 ITS-X. Stage 7 (day 22 and after):

10 μ M ZnSO₄, 10 μ g/mL heparin, 1:200 ITS-X, 1:1000 Trace Elements A (BD Sciences, #25-021-CI), 1:1000 Trace Elements B (BD Sciences, #25-022-CI), 1% MEM nonessential amino acid (Thermo Fisher Scientific, #11140076).

Human Islet Report:

The islets in Column 1 were implanted into male IsletTester mice (500/mouse) and the data is presented in Figure 4A-E. The Islets in Column 2 were implanted into female IsletTester mice (500/mouse) and part of the data is presented in Figure 4F.

Islet preparation	1	2
MANDATORY INFORMATION		
Unique identifier	RRID: SAMN41789873	RRID: SAMN30486796
Donor age (years)	60	50
Donor sex (M/F)	Male	Female
Donor BMI (kg/m ²)	27.4	31.01
Donor HbA _{1c} or other measure of blood glucose control	5.7	6.6
Origin/source of islets	IIDP	HPAP
Islet isolation centre	Imagine	UPenn
Donor history of diabetes? Please select yes/no from drop down list	No	No
RECOMMENDED INFORMATION		
Donor cause of death	Cerebrovascular/stroke	Cerebrovascular/stroke
Warm ischaemia time (h)	None	None
Cold ischaemia time (h)	8 hrs 34 min	10 hrs 14 min
Estimated purity (%)	67	NA
Estimated viability (%)	70	NA
Total culture time (h)	3 days 20 hours	NA
Glucose-stimulated insulin secretion or other functional measurement	Stimulus – Insulin AUC (ng/100 IEQ) G16.7 – 28.6 G16.7+IBMX 100 – 27.6 KCI 20 – 13.1	NA

Handpicked to purity? Please select yes/no from drop down list	Yes	Yes
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