Integrated human pseudoislet system and microfluidic platform demonstrates differences in G-protein-coupled-receptor signaling in islet cells

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Supplemental Information

Figure S1

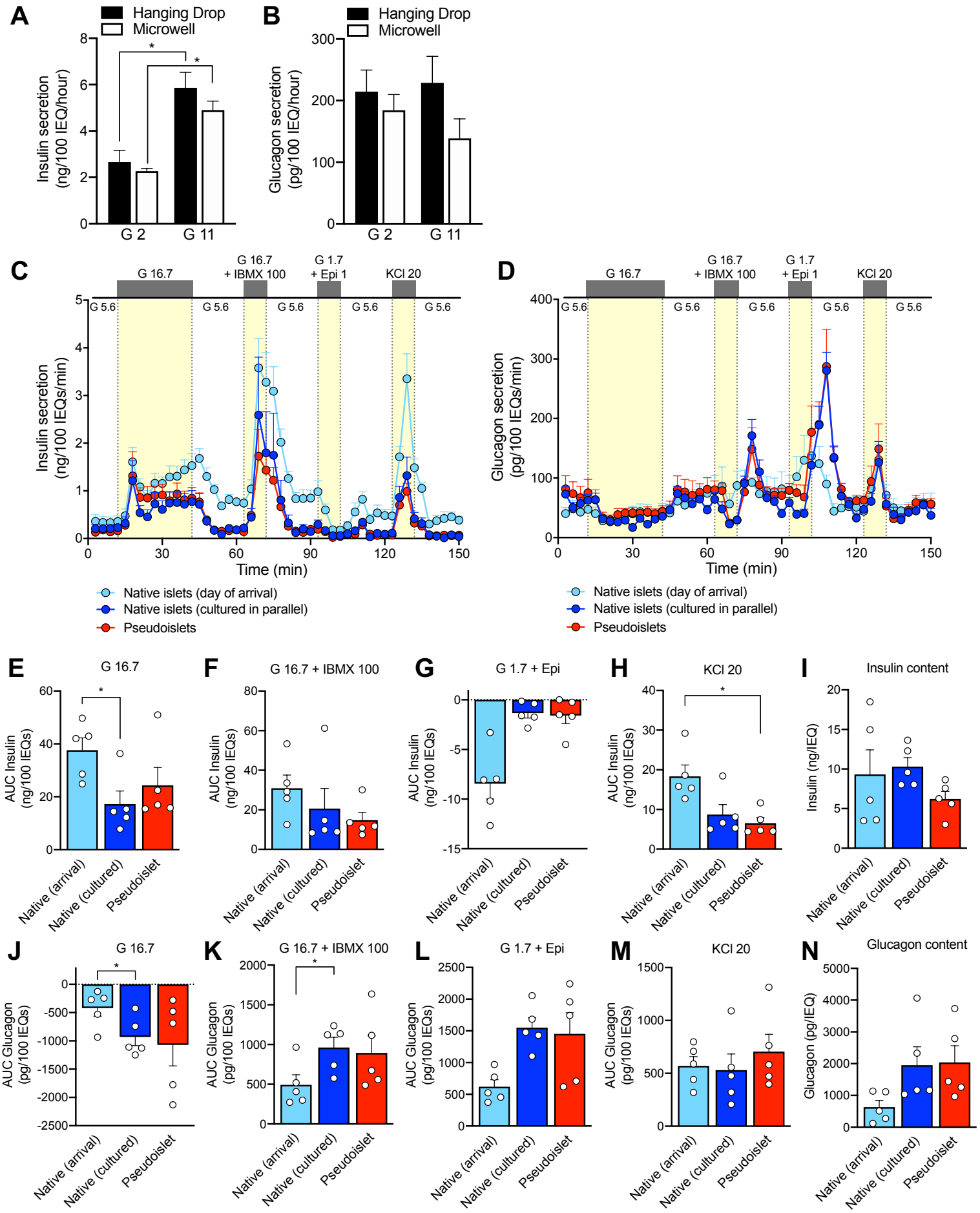

A
Hanging Drop
Microwell
Insulin secretion (ng/100 IEQ/hour)
G 2
G 11
B
Hanging Drop
Microwell
Glucagon secretion (pg/100 IEQ/hour)
G 2
G 11
C
G 16.7
G 16.7 + IBMX 100
G 1.7 + Epi 1
KCl 20
G 5.6
Insulin secretion (ng/100 IEQs/min)
Time (min)
Native islets (day of arrival)
Native islets (cultured in parallel)
Pseudoislets
D
G 16.7
G 16.7 + IBMX 100
G 1.7 + Epi 1
KCl 20
G 5.6
Glucagon secretion (pg/100 IEQs/min)
Time (min)
Native islets (day of arrival)
Native islets (cultured in parallel)
Pseudoislets
E
G 16.7
AUC Insulin (ng/100 IEQs)
Native (arrival)
Native (cultured)
Pseudoislet
F
G 16.7 + IBMX 100
AUC Insulin (ng/100 IEQs)
Native (arrival)
Native (cultured)
Pseudoislet
G
G 1.7 + Epi
AUC Insulin (ng/100 IEQs)
Native (arrival)
Native (cultured)
Pseudoislet
H
KCl 20
AUC Insulin (ng/100 IEQs)
Native (arrival)
Native (cultured)
Pseudoislet
I
Insulin content
Insulin (ng/IEQ)
Native (arrival)
Native (cultured)
Pseudoislet
J
G 16.7
AUC Glucagon (pg/100 IEQs)
Native (arrival)
Native (cultured)
Pseudoislet
K
G 16.7 + IBMX 100
AUC Glucagon (pg/100 IEQs)
Native (arrival)
Native (cultured)
Pseudoislet
L
G 1.7 + Epi
AUC Glucagon (pg/100 IEQs)
Native (arrival)
Native (cultured)
Pseudoislet
M
KCl 20
AUC Glucagon (pg/100 IEQs)
Native (arrival)
Native (cultured)
Pseudoislet
N
Glucagon content
Glucagon (pg/IEQ)
Native (arrival)
Native (cultured)
Pseudoislet

Figure S2

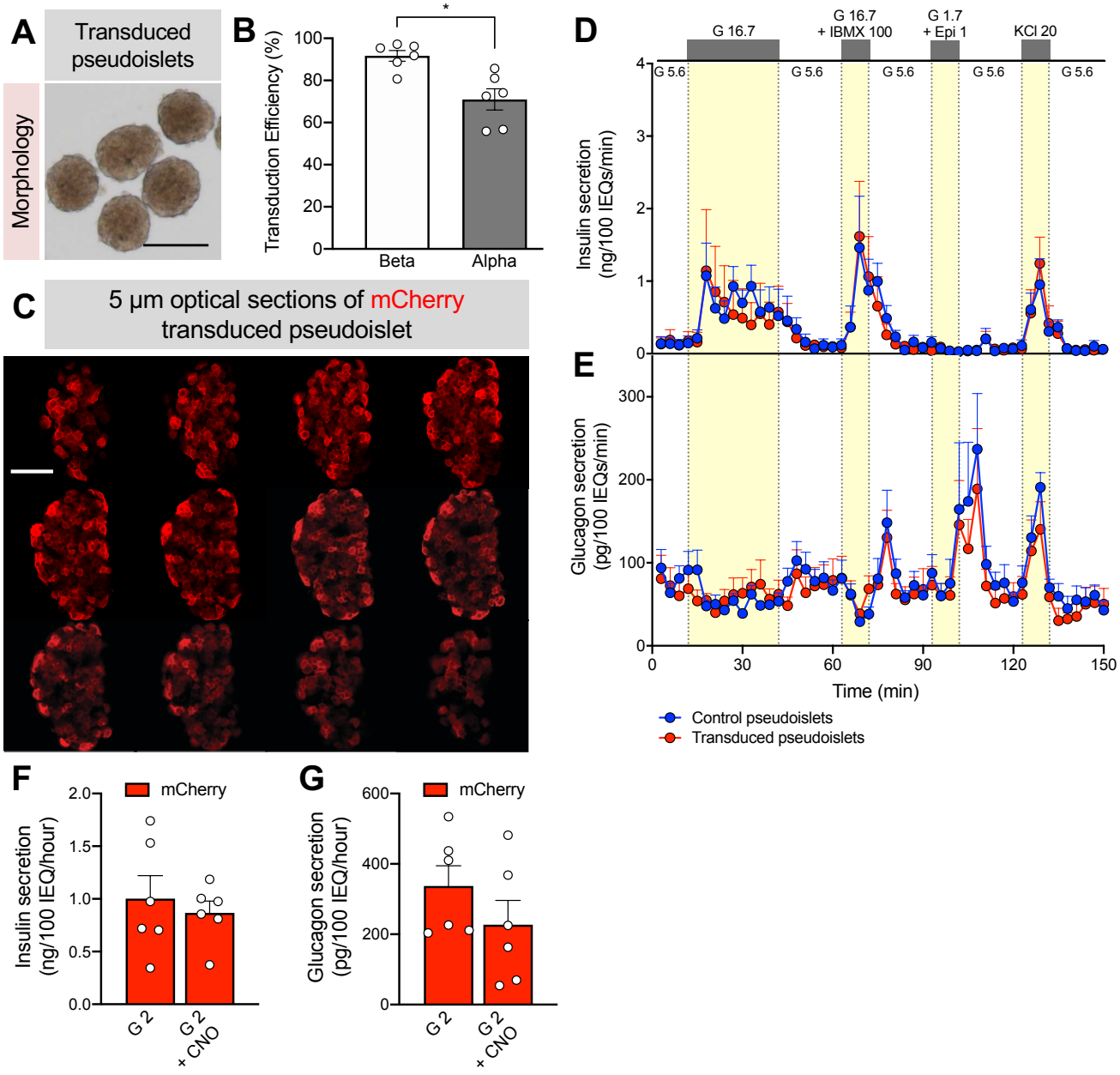

A
Transduced pseudoislets
Morphology
B
Transduction Efficiency (%)
100
80
60
40
20
0
*
Beta
Alpha
C
5 µm optical sections of mCherry transduced pseudoislet
D
G 16.7
G 16.7 + IBMX 100
G 1.7 + Epi 1
KCl 20
G 5.6
Insulin secretion (ng/100 IEQs/min)
E
Glucagon secretion (pg/100 IEQs/min)
Time (min)
Control pseudoislets
Transduced pseudoislets
F
mCherry
Insulin secretion (ng/100 IEQ/hour)
G 2
G 2 + CNO
G
mCherry
Glucagon secretion (pg/100 IEQ/hour)

Figure S3

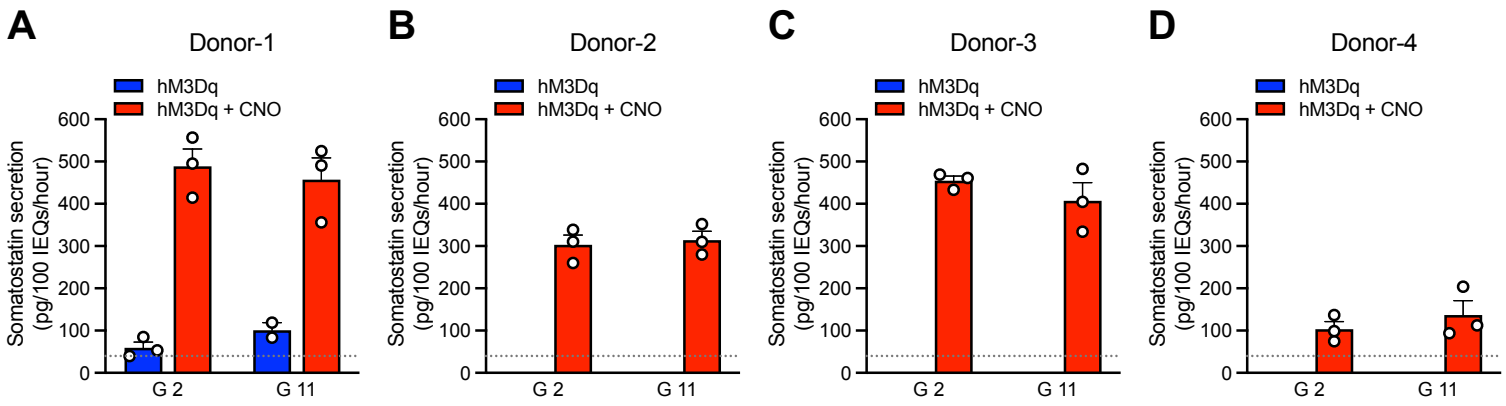
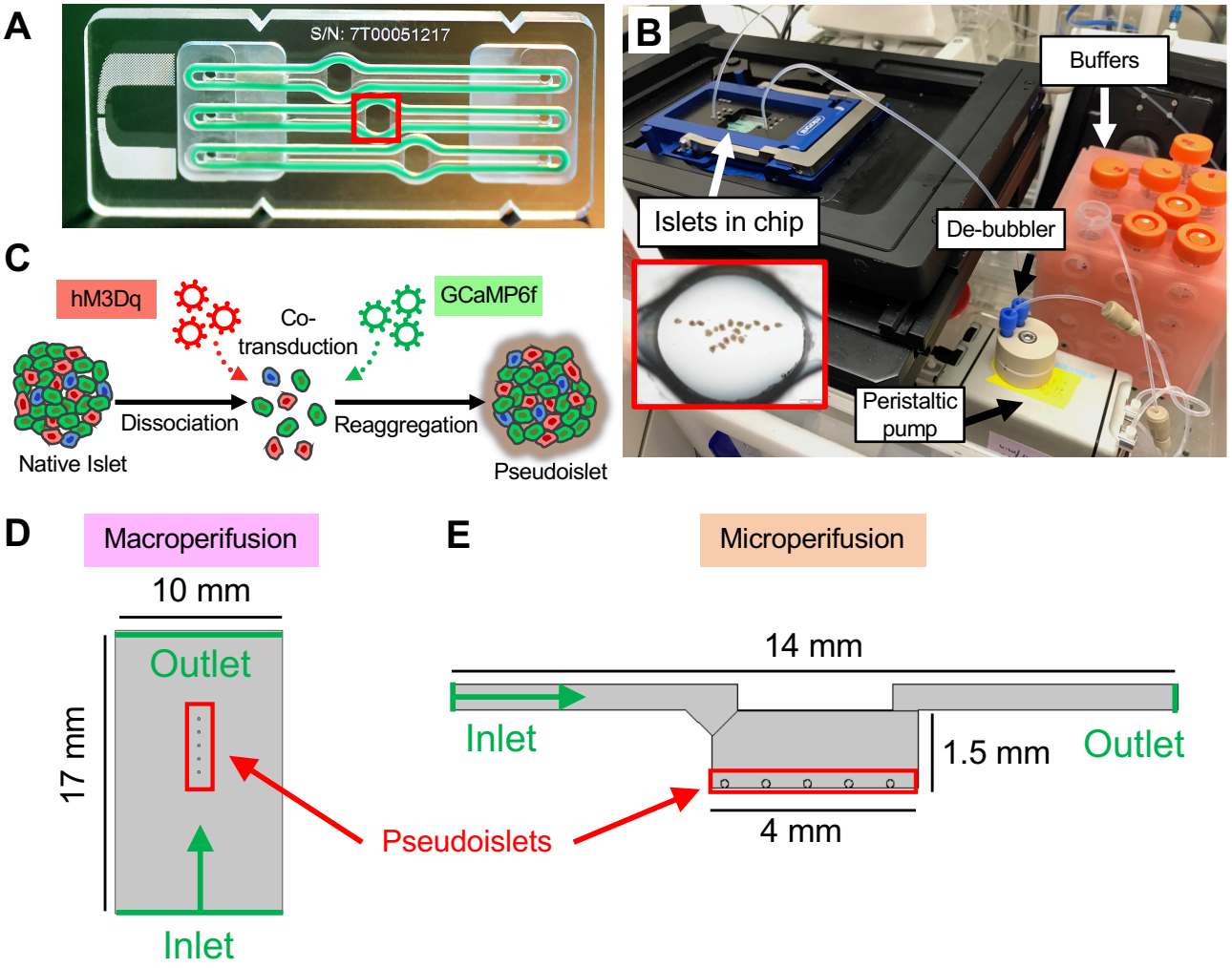
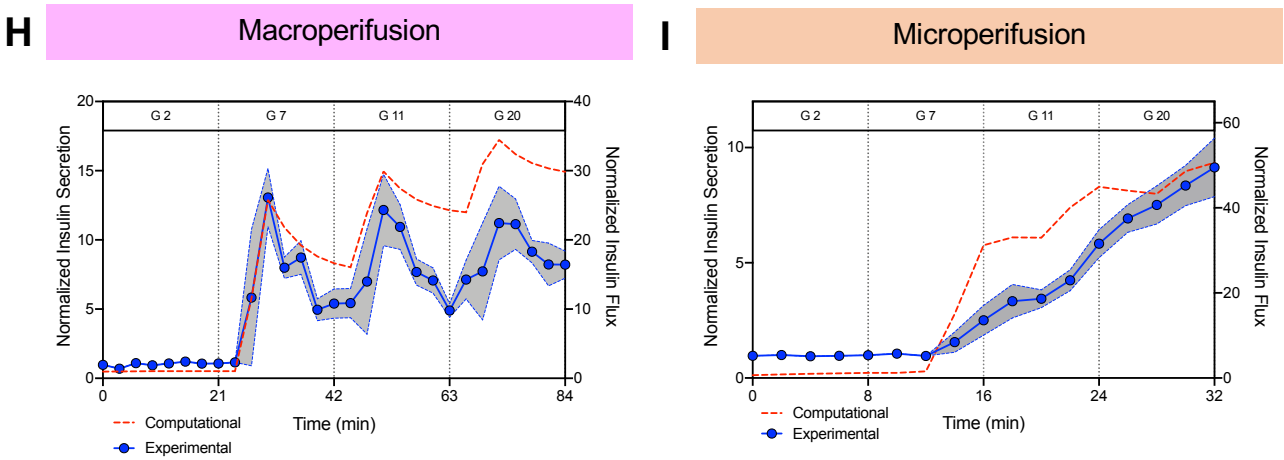

A
Donor-1
B
Donor-2
C
Donor-3
D
Donor-4
hM3Dq
hM3Dq + CNO
Somatostatin secretion (pg/100 IEQs/hour)
0
100
200
300
400
500
600
G 2
G 11

Figure S4



F

Perifusion parameters	Macro	Micro
Chamber volume (µL)	1500	65
Flow rate (µL/min)	3000	100
Fraction time (mins)	3	2
Stimulus time (mins)	21	8
Islet equivalents	~250	~25



SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Related to Figure 1. Evaluation of hormone secretory responses in pseudoislet system. Comparison of insulin (A) and glucagon secretion (B) by static incubation in pseudoislets made via modified hanging drop system (InSphero) versus ultra-low attachment microwell system (Perkin Elmer); n=1 donor, 3 replicates, $p > 0.05$. Insulin (C) and glucagon secretion (D) was measured by macroperifusion in native islets on the day of arrival to Vanderbilt (light blue trace) was compared in the same donor with secretory response of native islets cultured for six days in Vanderbilt pseudoislet media (dark blue trace, also shown in Figures 1H and 1I) and pseudoislets collected at the end of six day reaggregation period (red trace, also shown in Figures 1H and 1I); n=5 donors; G 5.6 – 5.6 mM glucose; G 16.7 – 16.7 mM glucose; G 16.7 + IBMX 100 – 16.7 mM glucose with 100 µM isobutylmethylxanthine (IBMX); G1.7 + Epi 1 – 1.7 mM glucose and 1 µM epinephrine (Epi); KCl 20 – 20 mM of potassium chloride (KCl). The area under the curve (AUC) of the insulin secretory responses to G 16.7 (E), G 16.7 + IBMX 100 (F), G 1.7 +Epi 1 (G), KCl 20 (H) and insulin content (E). The AUC of the glucagon secretory responses to G 16.7 (J), G 16.7 + IBMX 100 (K), G1.7 + Epi 1 (L), and KCl 20 (M), and glucagon content (N). One-way ANOVA with Dunn's multiple comparison test was used to analyze differences in panels E-N; *, $p<0.05$.

Figure S2. Related to Figure 3. Pseudoislet system allows for highly efficient transduction of human islet cells. (A) Bright-field image of transduced pseudoislets. Scale bar is 200 µm. (B) Transduction efficiency of β and α cells in this system; *, $p < 0.05$. (C) Confocal image of mCherry-transduced pseudoislet showing optical sections taken every 5-µm to highlight transduced cells throughout the entire pseudoislet. Scale bar is 100 µm. Insulin (D) and glucagon (E) secretion in response to a series of β and α cell secretagogues measured by macroperifusion in control pseudoislets and pseudoislets transduced with m-Cherry virus from the same donor (n=3 donors). Insulin (F) and glucagon secretion (G) in mCherry-expressing

pseudoislets measured in 2 mM glucose (G 2) with and without CNO (n=2 donors; n=3 replicates/donor; $p > 0.05$). Panels D and E were analyzed by 2-way ANOVA; $p > 0.05$. Panels F and G were compared by Mann-Whitney test.

Figure S3. Related to Figure 4. Somatostatin secretion from hM3Dq-expressing pseudoislets by static incubation. Somatostatin secretion in static incubation experiments at low (G 2; 2 mM glucose) and high (G 11; 11 mM glucose) with and without activation of G_q signaling (±10 μM CNO) across 4 independent donors (3 biological replicates/condition/donor other than Donor-1, G 11 which has only 2 replicates). Gray dotted line represents the limit of the assay sensitivity; somatostatin secretion in G 2 and G 11 for Donors 2-4 were below the limit of detection.

Figure S4. Related to Figure 5. Microperifusion system assembly and fluid dynamic modeling of macro- and microperifusion. (A) Picture of microfluidic device showing where islets are loaded and imaged (red box). There are three potential chambers to load islets, but in the current experimental layout, islets are loaded only into one chamber. (B) Experimental set-up of the microfluidic device on the confocal microscope stage within incubator including peristaltic pump, de-bubbler, and perifusion buffers. (C) Schematic of experimental workflow with incorporation of genetically encoded biosensor into hM3Dq-expressing pseudoislets. Schematic of the macroperifusion (D) and microperifusion chamber (E) showing the path of fluid flow. (F) Key experimental parameters of macroperifusion and microperifusion system. (H and I) Comparison of normalized insulin secretion acquired experimentally versus predicted by modeling in macroperifusion (H) and microperifusion (I). Experimental insulin data was normalized to average value in 2 mM glucose. The gray region demonstrates the SEM comparing experimental insulin secretion data and insulin flux from COMSOL computational

modeling in the macroperifusion system (H) and microperifusion system (I); G 2 – 2 mM, G 7 – 7 mM, G 1 – 11 mM, G 20 – 20 mM glucose.

Checklist for reporting human islet preparations used in research
Adapted from Hart NJ, Powers AC (2019) Progress, challenges, and suggestions for using human islets to understand islet biology and human diabetes. Diabetologia https://doi.org/10.1007/s00125-018-4772-2 and Poitout V, Satin LS, Kahn SE, et al (2019) A call for improved reporting of human islet characteristics in research articles. Diabetologia and Diabetes. https://doi.org/10.2337/dbi18-0055

Supplemental Table 1. Human Islet Donor Information

Islet preparation	1	2	3	4	5	6	7	8
Unique identifier	DON308	08619527	08617638	10861888	11046361	11106978	11578698	12713942
Donor age (years)	35	28	49	58	57	20	57	41
Donor sex	M	M	M	F	M	F	M	M
Donor BMI (kg/m^2)	24.7	34.7	34.0	36.6	35.9	36.9	25.8	23.5
Donor HbA1C	5.3	N/A	6.1	5.3	5.4	5.3	5.7	5.2
Origin/source of islets	AHN	IIDP	IIDP	IIDP	IIDP	IIDP	IIDP	IIDP
Islet isolation centre	AHN	SL	SC	SC	SL	SL	SL	SL
Donor history of diabetes?	No	No	No	No	No	No	No	No
Donor cause of death	Head Trauma	Head Trauma	Stroke	Anoxia	Stroke	Head Trauma	Stroke	Stroke
Warm ischaemia time (h)	N/A	N/A	N/A	0.3	N/A	N/A	N/A	N/A
Cold ischaemia time (h)	N/A	14.2	8.5	6.4	9.2	6.5	6.8	9.8
Estimated purity (%)	50	95	80	80	80	83	95	85
Estimated viability (%)	65	95	97	97	95	95	95	95
Total culture time (h)	80	103	47	96	120	92	114	81
Glucose-stimulated insulin secretion or other functional measurement	perifusion	perifusion	perifusion	perifusion	perifusion	perifusion	perifusion	perifusion
Handpicked to purity?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

Islet preparation	9	10	11	12	13	14	15	16
Unique identifier	10490796	10516338	R252	R253	R260	R264	R282	R286
Donor age (years)	53	52	26	57	73	44	57	41
Donor sex	F	F	F	M	F	M	M	M
Donor BMI (kg/m^2)	26.3	26.9	25.4	25.5	26.9	33.7	26.4	20.3
Donor HbA1C	5.4	4.5	5.0	5.0	6.2	5.7	6.0	5.2
Origin/source of islets	IIDP	IIDP	ADI Islet Core	ADI Islet Core	ADI Islet Core	ADI Islet Core	ADI Islet Core	ADI Islet Core
Islet isolation centre	SL	SL	ADI Islet Core	ADI Islet Core	ADI Islet Core	ADI Islet Core	ADI Islet Core	ADI Islet Core
Donor history of diabetes?	No	No	No	No	No	No	No	No
Donor cause of death	Anoxia	Stroke	N/A	N/A	N/A	N/A	N/A	N/A
Warm ischaemia time (h)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Cold ischaemia time (h)	7.6	6.3	11	14.3	11.5	11	9.5	13.5
Estimated purity (%)	90	80	85	90	80	90	90	95
Estimated viability (%)	95	95	N/A	N/A	N/A	N/A	N/A	N/A
Total culture time (h)	88	108	55	88	70	98	96	44
Glucose-stimulated insulin secretion or other functional measurement	perifusion	perifusion	perifusion	perifusion	perifusion	perifusion	perifusion	perifusion
Handpicked to purity?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

Islet preparation	17	18	19	20	21	22	23	24
Unique identifier	R306	R309	R314	R318	R323	R340	HPAP022	HPAP035
Donor age (years)	22	47	31	54	60	36	39	35
Donor sex	F	F	F	M	F	M	F	M
Donor BMI (kg/m^2)	21.1	27.4	30.3	20.5	24.9	23.3	34.76	26.91
Donor HbA1C	5.3	5.5	5.0	5.0	5.1	5.3	4.7	5.2
Origin/source of islets	ADI Islet Core	ADI Islet Core	ADI Islet Core	ADI Islet Core	ADI Islet Core	ADI Islet Core	HPAP	HPAP
Islet isolation centre	ADI Islet Core	ADI Islet Core	ADI Islet Core	ADI Islet Core	ADI Islet Core	ADI Islet Core	UPenn	UPenn
Donor history of diabetes?	No	No	No	No	No	No	No	No
Donor cause of death	N/A	N/A	N/A	N/A	N/A	N/A	Anoxia	Anoxia
Warm ischaemia time (h)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Cold ischaemia time (h)	15	11	14.75	16	14.5	12.5	8.55	12.9
Estimated purity (%)	80	90	80	90	90	95	95	90
Estimated viability (%)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Total culture time (h)	55	108	72	85	90	65	72	55
Glucose-stimulated insulin secretion or other functional measurement	perifusion	perifusion	perifusion	perifusion	perifusion	perifusion	perifusion	perifusion
Handpicked to purity?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

AHN – Allegheny Health Network, IIDP – Integrated islet distribution program, SL – The Scharp-Lacy Research Institute. SC – Southern California Islet Cell Resource Center, ADI IsletCore – Alberta Diabetes Institute IsletCore, HPAP – Human Pancreas Analysis Program,

Supplemental Table 2. Antibody Information

Antigen	Host Species	Dilution	Vendor/Source	Catalog #
ARX	Sheep	1:1000	R&D Systems	AF7068
C-peptide	Rat	1:1000	DSHB	GN-ID4
Caveolin-1	Rabbit	1:2000	Abcam	ab2910
Collagen-IV	Rabbit	1:1000	Rockland	600-401-106S
GFP	Chicken	1:1000	Abcam	ab13970
Glucagon	Rabbit	1:200	Cell Signaling	2760
Glucagon	Mouse	1:500	Abcam	ab10988
Insulin	Guinea pig	1:1000	Dako	A0564
Ki67	Rabbit	1:5000	Abcam	ab15580
MAFB	Rabbit	1:3000	Roland Stein*	N/A
mCherry	Rabbit	1:1000	Abcam	ab167453
NKX2.2	Mouse	1:1000	DSHB	74-5A5
NKX6.1	Rabbit	1:2000	BCBC/Palle Serup^	N/A
PAX6	Rabbit	1:5000	Covance	PRB-28P-100
PDX1	Rabbit	1:5000	C. V. E. Wright#	N/A
Somatostatin	Goat	1:500	Santa Cruz	sc-7819
VEGFR2	Goat	1:200	R&D Systems	AF644

BCBC – Beta Cell Biology Consortium, DHSB – Developmental Studies Hybridoma Bank, N/A – not applicable

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#Christopher Wright, Vanderbilt University, Nashville, TN, USA

Supplemental Table 3. Computational Modeling Parameters

	Parameter	Macroperifusion	Microperifusion
Fluid Dynamics	Fluid	Water	
	Density	993 kg/m^3	
	Dynamic Viscosity	$7x10^{-4}$ Pa/s	
	Inlet Velocity	$2.13x10^{-4}$ m/s	$3.34x10^{-2}$ m/s
Mass Transport	Diffusion Coefficient, Glucose in Fluid	$9x10^{-10}$ m^2/s	
	Diffusion Coefficient, Glucose in Islets	$3x10^{-10}$ m^2/s	
	Diffusion Coefficient, Oxygen in Fluid	$3x10^{-9}$ m^2/s	
	Diffusion Coefficient, Oxygen in Islets	$2x10^{-9}$ m^2/s	
	Diffusion Coefficient, Insulin in Fluid	$1.5x10^{-10}$ m^2/s	
	Diffusion Coefficient, Insulin in Islets	$0.5x10^{-10}$ m^2/s	
	Oxygen Concentration	0.2 mol/m^3	
Islet Physiology	Number of Islets	5	
	Radius	$7.5x10^{-5}$ m	
	Maximum Oxygen Consumption Rate	-0.034 $mol/s/m^3$	
	Maximum Glucose Consumption Rate	-0.028 $mol/s/m^3$	
	Insulin Release Rate Constant	$3x10^{-3}$ 1/s	
	Maximum First Phase Insulin Secretion Rate	$10x10^{-5}$ $mol/s/m^3$	
	Maximum Second Phase Insulin Secretion Rate	$1.8x10^{-5}$ $mol/s/m^3$	

*Parameters in center apply to both perifusion systems

Vanderbilt Pseudoislet Protocol

Materials and Reagents

- CellCarrier Spheroid ULA 96-well microplates (PerkinElmer, 6055330)
- PBS, Ca^{2+}/Mg^{2+} free (Gibco, 14190-144)
- 0.5 M EDTA, pH 8.0 (Cellgro, 46-034-CI)
- 0.25% HyClone™ Trypsin (Thermo Scientific, SH30042.01)
- CMRL 1066 (MediaTech, 15-110-CV)
- L-Glutamine 200 mM (Gibco, 25030-081)
- Fetal Bovine Serum, Heat Inactivated (Sigma-Aldrich, 12306C)
- Penicillin/Streptomycin (Gibco, 15140-122)
- 100 mM Sodium Pyruvate (Thermo Scientific, 11360070)
- 200 mM GlutaMAX-I (100X) Supplement (Gibco, 35050061)
- 1 M HEPES (Gibco, Life Technologies, 15630-080)
- 250 µg/mL Amphotericin B (Fisher, BP2645-20)
- VascuLife VEGF Medium Complete Kit (LifeLine Cell Technology, LL-0003)
- iCell Endothelial Cells Medium Supplement (Cell Dynamics, M1019)
- Non-treated, suspension cell culture dish (Sarstedt, 83.3900.500, 83.3901.500, 83.3902.500)

Equipment

- Centrifuge (Eppendorf, 5702R)
- Countess II (Life Technologies, AMAX1000)
- Inverted microscope (Olympus, CKX53)
- Stereomicroscope (Olympus, SZX12)
- Cell culture incubator (Fisher, Heracell)
- Laminar flow cabinet

Recipes

Standard CMRL 1066 Media (10% FBS, 100 µg/mL streptomycin, 100 U/mL penicillin, 2 mM L-Glutamine), 500 mL

CMRL 1066	440 mL
HI-FBS	50 mL
Pen/Strep	5 mL
L-Glutamine	5 mL

2mM EDTA, 10 mL

0.5M EDTA	40 µL
PBS, Ca^{2+}/Mg^{2+} free	10 mL

*Has a 1-month shelf life at 4°C

0.025% Trypsin, 1 mL

0.25% Trypsin	100 µL
PBS, Ca^{2+}/Mg^{2+} free	900 µL

*****Make fresh each time

Enriched CMRL 1066 Media (20% FBS, 100 µg/mL streptomycin, 100 U/mL penicillin, 1 mM sodium pyruvate, 2 mM Glutamax, 2 mM HEPES), 200 mL

CMRL 1066	154 mL
HI-FBS	40 mL
Pen/Strep	2 mL
GlutaMAX	2 mL
HEPES	400 µL
Sodium Pyruvate	2 mL

*Combine and filter sterilize through a 0.22 µm filter. Has a 1-month shelf-life at 4°C.

iEC (VascuLife Endothelial Mix + FBS from iEC Cell Dynamics), 500 mL

VascuLife Basal Medium	436 mL
rh VEGF LifeFactor	0.5 mL
rh EGF LifeFactor	0.5 mL
rh FGF basic LifeFactor	0.5 mL
rh IGF-1 LifeFactor	0.5 mL
Ascorbic Acid LifeFactor	0.5 mL
Hydrocortisone Hemisuccinate LifeFactor	0.5 mL
Heparin Sulfate LifeFactor	0.5 mL
Gentamicin/Amphotericin B	0.5 mL
L-glutamine LifeFactor	10 mL
iCell Endothelial Cells Medium Supplement	50 mL

*Combine filter sterilize through a 0.22 µm filter. Has a 1-month shelf-life at 4°C.

Vanderbilt Pseudoislet Media (VPM): Combine equal volumes of “Enriched-CMRL” and “iEC” media

Protocol

Request (Day 0) and Handpick Human Islets (Day 1)

1. Request appropriate number of IEQs. Assess islet quality by considering donor attributes (age, BMI, HbA1C), islet isolation center, culture time (<2 days), and available islet images. We typically handpick approximately 1,000-1,500 islets which will generate approximately 700 pseudoislets.
2. On the day of arrival, perform appropriate quality control studies. We use islet imaging (brightfield, DTZ, FDA/PI staining), dispersed islet cell viability, and islet functional assessment by perifusion. For islets distributed by the Integrated Islet Distribution Program (IIDP) beginning July 2016, the islet phenotyping information is now available to islet recipients through the Human Islet Phenotyping Program database viewable in the IIDP portal (https://iidp.coh.org/secure/isletavail/default.aspx).
3. Handpick desired number islets to high purity into 6-cm non-treated Sarstedt dishes containing standard CMRL media. Assume a medium-sized islet will produce approximately 1000 cells, so you will need to pick 2-3 times the number of desired pseudoislets.
4. Collect appropriate number of islets that will remain intact for parallel long-term culture as native controls.
5. Culture islets in standard CMRL media in a humidified incubator at 37°C, 5% CO_2/95% air overnight.

Dissociation (Day 2)

6. Transfer islets for dispersion into sterile, clear 1.5-mL Eppendorf tube and wash 3 times with 1 mL of 2 mM EDTA in 1X PBS. For washes, centrifuge islets for 2-3 minutes at 4°C and 1000 rpm in benchtop Eppendorf centrifuge.
7. Using a P1000, disperse islets for 7 minutes at room temperature with 0.025% trypsin by pipetting slowly up and down to mix. You will notice the solution becomes opaque by visual inspection as islets break apart. Use 500 µL of 0.025% trypsin. If you have more than 500 islets, use additional 0.025% trypsin (100 µL per additional 100 islets).
8. Quench trypsin by adding an equal volume of VPM media then wash 2 times with 1 mL of VPM media. For washes, centrifuge dispersed cells for 2-3 minutes at 4°C and 1800 rpm in a benchtop Eppendorf centrifuge.
9. Resuspend in defined volume of VPM media (typically use 1 mL) for counting.

Cell Count and Reaggregation (Day 2)

10. Count cell density manually using hemacytometer or with an automated Countess II cell counter to determine cell count and viability. For Countess II, add 10 µL of Trypan Blue to a 10 µL aliquot of the 1 mL cell suspension.
11. Use cell counts to determine number of pseudoislets for each group. We use 2000 cells/pseudoislet and generate pseudoislets between 150-250 µm in diameter.
12. Resuspend each group in appropriate volume (200 µL/pseudoislet) of VPM media.
13. Using a multichannel P200 pipettor, pipette the cell solution up and down to ensure homogeneity and pipette 200 µL of the cell suspension into each well of the CellCarrier ULA microwell plates.

14. Transfer native islet controls into appropriately sized non-treated Sarstedt culture dish (typically 6-cm) with VPM media.
15. Incubate the pseudoislets and native islet controls in humidified incubator at 37°C, 5% CO_2/95% air for 4 days without media changes.

Pseudoislet Medium Exchange (Day 6)

16. Exchange media by placing a multi-pipettor at a 45° angle into the wells and slowly aspirating off 100 µL (half) of media. Do not place all the way in the well or you will pull up pseudoislet.
17. Add 100 µL of fresh VPM media to each well.
18. Using a microscope, visually inspect pseudoislets and ensure pseudoislets are still present in wells.
19. Exchange media of native islets by picking into a clean plate of VPM media.
20. Incubate the pseudoislets and native islets in a humidified incubator at 37°C, 5% CO_2/95% air overnight.

Pseudoislet Harvest Plate (Day 8)

21. Add 1mL of VPM media to 6-cm Sarstedt dish to prepare for harvest. Media from aspirated wells will fill up the rest of the dish.
22. Set a multichannel (6 tips) pipette to 200 µL and pre-wet tip with VPM media. With the plunger pressed, place the pipette tips along the side of the wells to keep the pipette tip opening clear.
23. Aspirate media and pseudoislets into tip and dispense into prepared 6-cm dish. You should remove approximately 190 µL of media.
24. After collecting from all wells, visually verify that pseudoislets have been removed from each well. If there are any left behind, add approximately 100 µL of VPM media to the well and repeat harvesting process.
25. Collected pseudoislets are ready for study. Handpick them into a clean plate after harvest to remove debris and bubbles if desired.

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