## ESM Methods

### Mouse islet isolation

Mice were sacrificed under isoflurane and their pancreases were perfused with Liberase solution (#5401020001, Sigma-Aldrich, Burlington, MA). After vigorous shaking, the mixture was centrifuged at 350 *g* at 4°C for 3 min and the supernatant was decanted. The pellet was resuspended with M199 media (Sigma Aldrich) and poured through a sieve. The mixture was re-centrifuged, and the supernatant decanted before supplying with cold Ficoll-Paque PLUS density gradient (Sigma-Aldrich) and M199 media without FBS. The mixture was re-centrifuged at 1610 *g* at 4°C for 22 min. The supernatant was collected, and pellet discarded and supplied with M199 media before centrifuging. Islets were allowed to settle by leaving the tube on ice for 4 min and then stained with dithizone dye (Cole-Parmer Scientific Experts, Vernon Hills, IL) to identify beta cells before counting. Islets were hand-picked with a 20 µl pipette under a stereomicroscope for further in-culture experiments prior or embedded into OCT.

### Measurement of corrected total cell fluorescence.

At least 3 regions-of-interest were obtained for every slide. Images were analysed for integrated density, area and mean grey value by selecting regions of interest using the selection tool in ImageJ. Background regions (no fluorescence) were also selected and analysed for the same. Size of area selected was kept consistent throughout analyses. From the values obtained, "Corrected Total Cell Fluorescence (CTCF)" was calculated on Excel using the formula: CTCF = Integrated Density – (Area of selected islet × Mean fluorescence of background readings). In brief, for each slide (n = 3) per group, a CTCF average of 3 images was obtained. CTCF immunofluorescence quantification has been published previously[1].

**Single Cell RNAseq analysis**

The dataset included normalized gene expression matrices and associated cell-type annotations generated by HPAP, encompassing major pancreatic cell populations (e.g., alpha, beta, ductal, endothelial, and immune cells) as previously reported[2-4]. All analyses, including clustering and visualization, were performed in R using the Seurat package unless otherwise indicated.

Quality control (e.g., filtering cells based on gene count or mitochondrial content) was carried out by HPAP, and these parameters were not further modified here. Principal component analysis (PCA) was employed for initial dimensionality reduction, followed by uniform manifold approximation and projection (UMAP) to generate two-dimensional embeddings. Clusters were reviewed to confirm alignment with the original HPAP-provided cell-type labels and not reassigned. Dot plot visualizations were created to illustrate relative gene expression and the proportion of cells in each cluster or sample group.

**References**

[1] Nourmohammadzadeh M, Xing Y, Lee JW, et al. (2016) A microfluidic array for real-time live-cell imaging of human and rodent pancreatic islets. Lab Chip 16(8): 1466-1472. 10.1039/c5lc01173f

[2] Kaestner KH, Powers AC, Naji A, Consortium H, Atkinson MA (2019) NIH Initiative to Improve Understanding of the Pancreas, Islet, and Autoimmunity in Type 1 Diabetes: The Human Pancreas Analysis Program (HPAP). Diabetes 68(7): 1394-1402. 10.2337/db19-0058

[3] Shapira SN, Naji A, Atkinson MA, Powers AC, Kaestner KH (2022) Understanding islet dysfunction in type 2 diabetes through multidimensional pancreatic phenotyping: The Human Pancreas Analysis Program. Cell Metab 34(12): 1906-1913. 10.1016/j.cmet.2022.09.013

[4] Patil AR, Schug J, Naji A, Kaestner KH, Faryabi RB, Vahedi G (2023) Single-cell expression profiling of islets generated by the Human Pancreas Analysis Program. Nat Metab 5(5): 713-715. 10.1038/s42255-023-00806-x

**ESM Table 1: Human pancreas samples**

| Donor ID | Demographic data<br>Cause of death | Diagnosis |
|---|---|---|
| D21-1345<br>Figure 7C | F, 29 y, BMI 37.2<br>Cerebral hypoxia | T1D |
| | | |
| D22-0300<br>Figure 4B, 4C, 4D (image and measurements) | F, 55 y, BMI 28.6<br>Intracerebral haemorrhage | T2D |
| D24-0359<br>Figure 4C, 4D (measurements) | M, 58 y, BMI 28.1<br>Traumatic brain injury | T2D |
| D22-0665<br>Figure 4B (measurements) | F, 60y, BMI 34<br>Intracerebral haemorrhage | T2D |
| D22-0554<br>Figure 4B, 4C, 4D (measurements) | M, 59 y, BMI 26.3<br>CVA (MCA infarction) | T2D |
| | | |
| D22-0173<br>Figure 4C, 4D (measurements) | F, 19 y, BMI 22.4<br>Traumatic brain injury | Healthy donor, young |
| D22-0488<br>Figure 4B, 4C, 4D (measurements) | F, 13 y, BMI 19.2<br>Cerebral hypoxia | Healthy donor, young |
| D22-0485<br>Figure 4B (measurements) | M, 24 y, BMI 25.5<br>Motor vehicle accident | Healthy donor, young |
| D22-0397<br>Figure 4C, 4D (image and measurements) | M, 7 y, BMI 20<br>Intracerebral haemorrhage | Healthy donor, young |
| D22-0367<br>Figure 4B (image and measurements), 7C | F, 32 y, BMI 20.3<br>Cerebral hypoxia | Healthy donor, young |
| | | |
| D23-0254<br>Figure 4B, 4D (measurements) | M, 70 y, BMI 29.3<br>Intracerebral haemorrhage | Healthy donor, aged |
| D22-0871<br>Figure 4B (measurements) | F, 65 y, BMI 23.5<br>Cardiac arrest, cerebral hypoxia | Healthy donor, aged |
| D24-0645 | M, 63 y, BMI 26 | Healthy donor, aged |

| | | |
|---|---|---|
| Figure 4B (image and measurements) | Cardiac arrest, cerebral hypoxia | |
| D22-1431<br>Figure 4C (measurements) | M, 69 y, BMI 26.5<br>Intracerebral haemorrhage | Healthy donor, aged |
| D23-0607<br>Figure 4C, 4D (image and measurements) | M, 76 y, BMI 26.8<br>Traumatic brain injury | Healthy donor, aged |
| D23-1379<br>Figure 4C, 4D (measurements) | F, 79 y, BMI 36.9<br>Out of hospital cardiac arrest | Healthy donor, aged |
| D22-0871<br>Figure 4B (measurements) | F, 64 y, BMI 23.5<br>Out of hospital cardiac arrest, cerebral hypoxia | Healthy donor, aged |

**ESM Table 2: Primary antibodies**

| Antibody | Target | Dilution | Molecular Weight of protein (kDa) | Catalogue # | Source |
|---|---|---|---|---|---|
| Mouse α-CD47 (B6H12) | Human CD47 | SDS-PAGE (in blocking buffer): 1.5:1000<br>Blocking CD47 in cells/islets/tissue: 1μg/ml<br>Immunofluorescence (in 2% BSA): 1:150 | ~50 | sc-12730 | Santa Cruz Biotechnology, Dallas, TX |
| Rat α-CD47 (MIAP301) | Mouse CD47 | SDS-PAGE (in blocking buffer): 1.5:1000<br>Blocking CD47 in cells/islets/tissue: 1μg/ml<br>Immunofluorescence (in 2% BSA): 1:150 (tissues), 1:50 (cells) | ~50 | sc-12731 | Santa Cruz Biotechnology |
| Mouse α-Insulin (L6B10) | Mouse and Human | SDS-PAGE (in blocking buffer): 1:1000<br>Immunofluorescence (in 2% BSA): 1:150 (tissues) | ~6 | 8138S | Cell Signalling Technology, Danvers, MA |
| Rabbit α-Insulin (C27C9) | Mouse and Human | SDS-PAGE (in blocking buffer): 1:1000<br>Immunofluorescence (in 2% BSA): 1:150 (tissues) or 1:50 (cells) | ~6 | 3014S | Cell Signalling Technology |
| Rabbit α-TSP1 | Human and mouse TSP1 | SDS-PAGE (in blocking buffer): 1:1000 | ~155 | ab85762 | Abcam, Cambridge, UK |

| | | | | | |
|---|---|---|---|---|---|
| Mouse α-TSP1 | Human TSP1 | Immunofluorescence (in 2% BSA): 1:150 (tissues) | – | ab1823 | Abcam |
| Rabbit α-HIF-1α (EPR16897) | Human and mouse HIF-1α | SDS-PAGE (in blocking buffer): 1:1000 | ~110 | ab179483 | Cell Signalling Technology |
| Rabbit α-Bcl-2 (D17C4) | Human and mouse Bcl-2 | SDS-PAGE (in blocking buffer): 1.5:1000 | ~26 | 3498S | Cell Signalling Technology |
| Rabbit α-Bcl-XL (54H6) | Human and mouse Bcl-XL | SDS-PAGE (in blocking buffer): 1.5:1000 | ~30 | 2764S | Cell Signalling Technology |
| Rabbit α-Vinculin (E1E9V) XP® | Mouse and Human Vinculin | SDS-PAGE (in blocking buffer): 1:1000 | ~124 | 13901S | Cell Signalling Technology |
| Rabbit α-BiP | Mouse and Human BiP | SDS-PAGE (in blocking buffer): 1:1000 | ~78 | 3177S | Cell Signalling Technology |
| Rabbit α-phospho-(p)- eIF2α (Ser51) | Mouse and Human p-eIF2α | SDS-PAGE (in blocking buffer): 1:1000 | ~38 | 9721S | Cell Signalling Technology |
| Rabbit α-total eIF2α | Mouse and Human total-eIF2α | SDS-PAGE (in blocking buffer): 1:1000 | ~38 | 9722S | Cell Signalling Technology |
| Rabbit α-IRE1α (14C10) | Mouse and Human IRE1α | SDS-PAGE (in blocking buffer): 1:1000 | ~130 | 3294S | Cell Signalling Technology |
| Rabbit α-CHOP | Mouse CHOP | SDS-PAGE (in blocking buffer): 1:1000 | ~27 | 5554S | Cell Signalling Technology |
| Rabbit α-Atg5 (D5F5U) | Mouse/human Atg5 | SDS-PAGE (in blocking buffer): 1:1000 | ~55 | 12994S | Cell Signalling Technology |
| Rabbit α-Atg7 (D12B11) | Mouse/human Atg7 | SDS-PAGE (in blocking buffer): 1:1000 | ~78 | 8558S | Cell Signalling Technology |

| | | | | | |
|---|---|---|---|---|---|
| Rabbit α-Beclin-1 (D40C5) | Mouse/human Beclin-1 | SDS-PAGE (in blocking buffer): 1:1000 | ~60 | 3495S | Cell Signalling Technology |
| Rabbit α-p62 | Mouse/human p62 | SDS-PAGE (in blocking buffer): 1:1000 | ~60 | ab91526 | Abcam |
| Rabbit LC3A/B (D3U4C) | Mouse/human LC3 I/II | SDS-PAGE (in blocking buffer): 1:1000 | I ~14<br>II ~16 | 12741S | Cell Signalling Technology |
| Mouse α-β-actin (8H10D10) | Mouse/human β-actin | SDS-PAGE (in blocking buffer): 1:2500 | ~42 | 3700S | Cell Signalling Technology |
| Rabbit α-p16-INK4A | Human $p16^{INK4A}$ | Immunofluorescence (in 2% BSA): 1:100 | – | 10883-1-AP | Proteintech, Rosemont, IL |
| Rabbit α-p21 (EPR362) | Human $p21^{cip1}$ | Immunofluorescence (in 2% BSA): 1:100 | – | ab188224 | Abcam |
| Rat α-IgG2a (RMG2a-62) Isotype Control | Mouse IgG | Immunofluorescence (in 2% BSA): 1:150 (tissues) or 1:50 (cells) | – | sc-53758 | Santa Cruz Biotechnology |
| Mouse α-IgG Antibody (D-1) Isotype Control | Human IgG | Immunofluorescence (in 2% BSA): 1:150 (tissues) or 1:50 (cells) | – | sc-515946 | Santa Cruz Biotechnology |

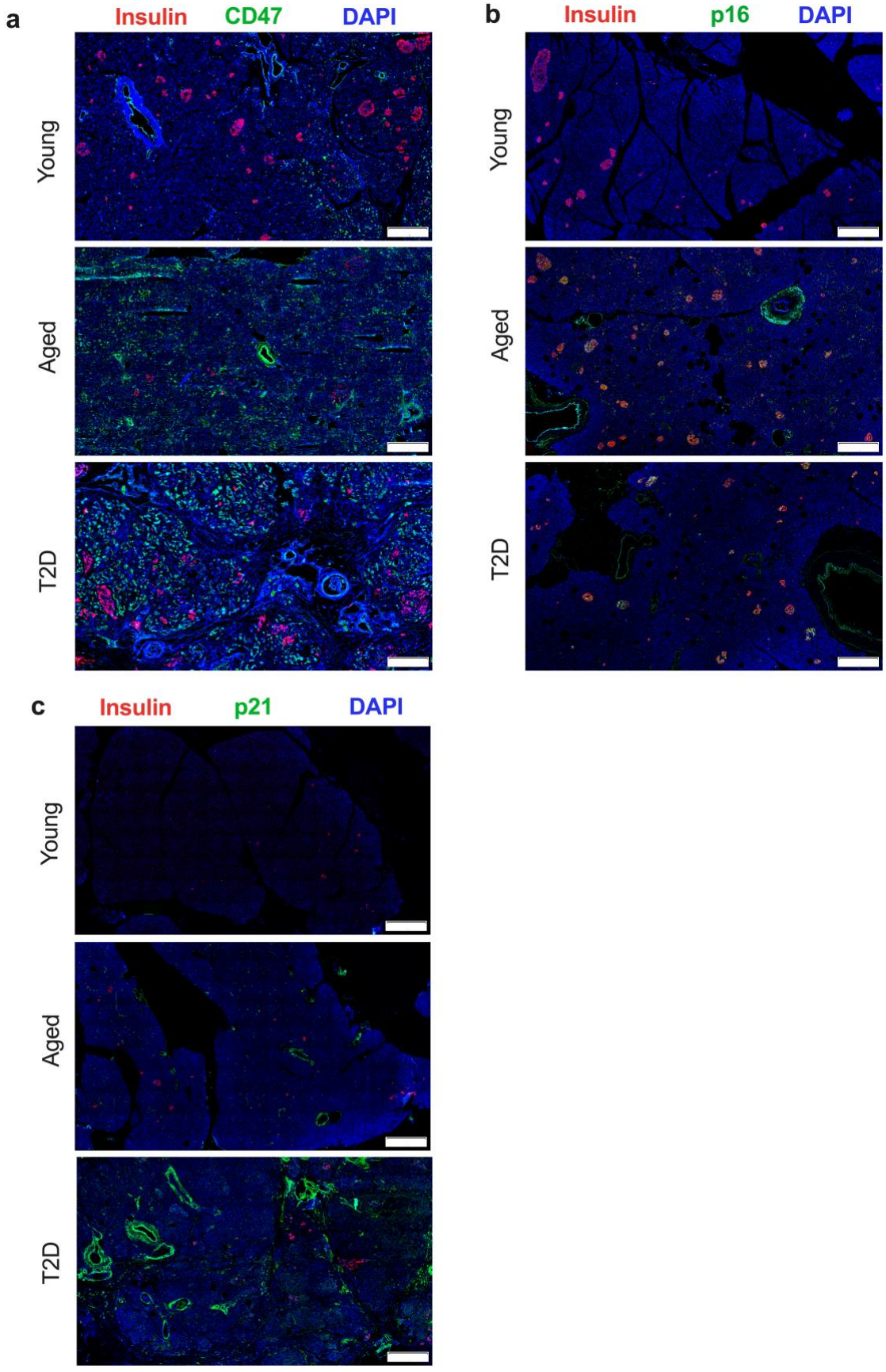
**ESM Table 3: Secondary Antibodies**

| Antibody | Dilution | Source |
|---|---|---|
| Alexa Fluor 488 \| 546 α-Mouse<br>Alexa Fluor 488 \| 546 α-Rabbit<br>Alexa Fluor 488 \| 546 α-Rat | For immunofluorescence: 1:100 (in PBS-T) | Invitrogen, Waltham, MA |
| Li-COR<br>Green \| Red α-Mouse<br>Green \| Red α-Rat<br>Green \| Red α-Rabbit | For SDS-PAGE: 1:10000 (in TBS-T) | Li-COR Biosciences, Lincoln, NE |

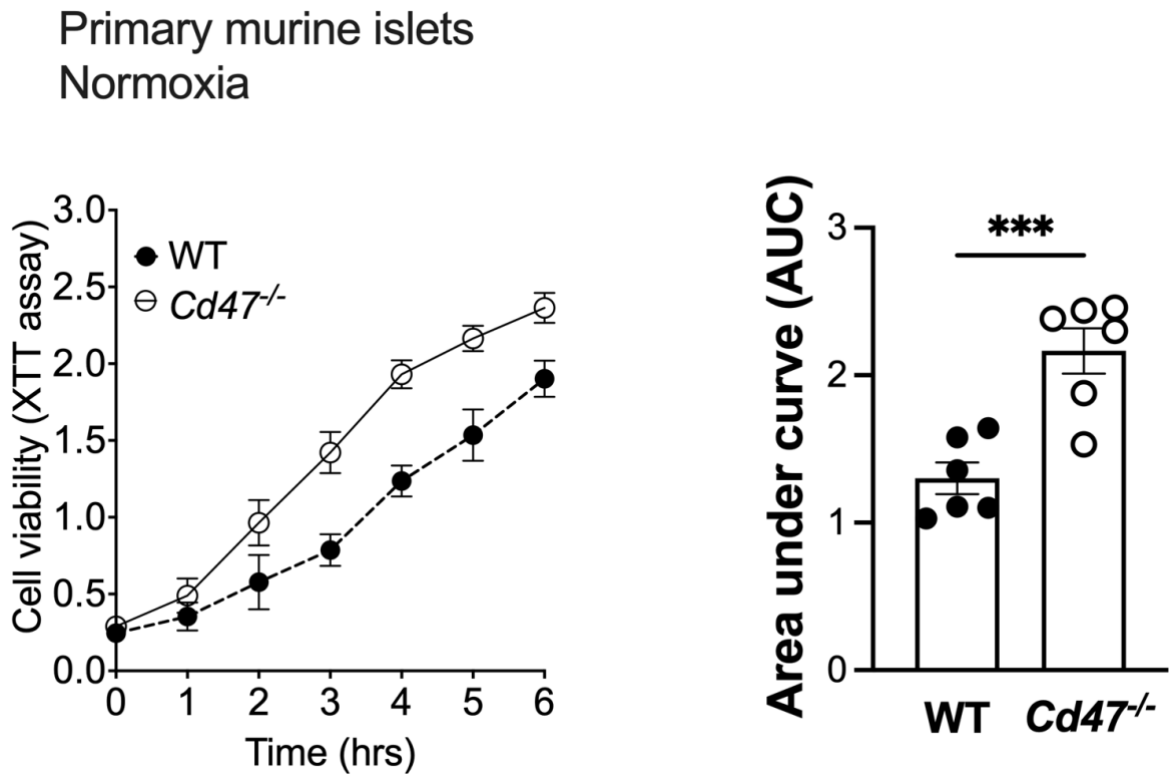
**ESM Table 4: Taqman Primers (ThermoFisher Scientific)**

| Gene | Assay ID |
|---|---|
| Rn18S | Mm03928990_g1 |
| Thbs1 | Mm00449032_g1 |
| CD47 | Mm00495011_m1 |
| Atg5 | Mm01187303_m1 |
| Atg7 | Mm00512209_m1 |
| Beclin-1 | Mm01265461_m1 |
| Sqstm1 (p62) | Mm0448091_m1 |
| Pou5f1 (Oct3/4) | Mm03053917_g1 |
| Sox2 | Mm03053810_s1 |
| Klf4 | Mm00516194_m1 |
| Myc | Mm00487804_m1 |
| Hspa5 (BiP) | Mm00517691_m1 |
| ERN1 (IRE1α) | Mm00470233_m1 |
| Ddit3 (CHOP) | Mm07294308_m1 |

**ESM Figure 1**


a
Insulin
CD47
DAPI
Young
Aged
T2D
b
Insulin
p16
DAPI
Young
Aged
T2D
c
Insulin
p21
DAPI
Young
Aged
T2D

**Senescence is upregulated by ageing and diabetes.** Human pancreas samples from non-diabetic young (n=5) and aged (n=6) organ donors, as well as donors with T2D (n=4) were stained for (a) insulin and CD47, (b) insulin and p16, as well as (c) insulin and p21. Images were obtained at 4x magnification. Scale bar: 500 µm.




**ESM Figure 2**

**CD47 signalling limits beta cell viability.** WT and CD47$^{-/-}$ murine islets (from n=3-5 samples isolated from n=3-4 mice/sample) were isolated and XTT viability assay was performed under standard culture conditions.

Diabetologia

# Checklist for reporting human islet preparations used in research

Adapted from Hart NJ, Powers AC (2018) 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

| **Islet preparation** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8[a]** |
|---|---|---|---|---|---|---|---|---|
| **MANDATORY INFORMATION** | | | | | | | | |
| Unique identifier | H250 (D17-0003 | H259 (D17-0314) | H263 (D17-0556) | H265 (D18-0065) | H298 (D21-0422) | H300 (D21-0510) | H304 (D22-1103) | H299 (D21-0448) |
| Donor age (years) | 44 | 55 | 47 | 57 | 47 | 48 | 44 | 28 |
| Donor sex (M/F) | M | F | M | F | F | F | M | M |
| Donor BMI (kg/m$^2$) | 30.86 | 20.24 | 27.68 | 23.31 | 44.41 | 23.44 | 24.4 | 30.19 |
| Donor $HbA_{1c}$ or other measure of blood glucose control | No insulin requirement, random BGL 8mmol/L | No insulin requirement, random BGL 8.2mmol/L | No insulin requirements, BGL 9.7mmol/L | No insulin requirements, BGL 7.3mmol/L | No insulin requirement, BGL<10mmol/L | No insulin requirement, random BGL 6.8mmol/L | No insulin requirement, random BGL 4.8mmol/L | No insulin requirement, BGL 16.3 |
| Origin/source of islets[b] | NSW | NSW | National Islet Transplant Consortium Australia | National Islet Transplant Consortium Australia | National Islet Transplant Consortium Australia | NSW | VIC | National Islet Transplant Consortium Australia |
| Islet isolation centre | Westmead Hospital | Westmead Hospital | Westmead Hospital | Westmead Hospital | Westmead Hospital | Westmead Hospital | Westmead Hospital | Westmead Hospital |
| Donor history of diabetes? Please select yes/no from drop down list | No | No | No | No | No | No | No | No |
| **If Yes, complete the next two lines if this information is available** | | | | | | | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Diabetes duration (years) | | | | | | | | |
| Glucose-lowering therapy at time of death[c] | | | | | | | | |
| **RECOMMENDED INFORMATION** | | | | | | | | |
| Donor cause of death | Hypoxic brain injury | Subarachnoid hemorrhage | Intracranial hemorrhage | Cerebral hypoxia | Cerebral hypoxia | Intracranial hemorrhage | Cerebral infarct | Traumatic brain injury |
| Warm ischaemia time (h) | Not available | Not available | Not available | Not available | Not available | Not available | Not available | |
| Cold ischaemia time (h) | 14.6 | 12.83 | 8.7 | 24.7 | 26.2 | 11.54 | 20.2 | 20.1 |
| Estimated purity (%) | 75% | 60% | 60% | 80% | 80% | 70% | 70% | 50% |
| Estimated viability (%) | 82.6% | 95% | 100% | 95% | 80% | 90% | 81.5% | 80% |
| Total culture time (h)[d] | <24 h | <24 h | <24 h | <24 h | <24 h | <24 h | <24 h | <24 h |
| Glucose-stimulated insulin secretion or other functional measurement[e] | Stimulation index 1.15 | Stimulation index 2.12 | Stimulation index 1.01 | Stimulation index 1.17 | Not performed | Not performed | Not performed | Not performed |
| Handpicked to purity? Please select yes/no from drop down list | No | No | No | No | No | No | No | No |
| Additional notes | Used in Figures 1A, 1B | Used in Figures 1A, 1B | Used in Figures 1A, 1B | Used in Figure 1B | Used in Figures 1A, 1C, 1D, 5F, 7A, 7B | Used in Figures 1A, 1C, 1D, 5F | Used in Figures 1A, 1C, 1D, 5F, 7A, 7B | Used in Figures 1C, 1D, 5F, 7A, 7B |

[a]If you have used more than eight islet preparations, please complete additional forms as necessary

[b]For example, IIDP, ECIT, Alberta IsletCore

[c]Please specify the therapy/therapies

[d]Time of islet culture at the isolation centre, during shipment and at the receiving laboratory

[e]Please specify the test and the results

| Islet preparation | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8[a] |
|---|---|---|---|---|---|---|---|---|
| **MANDATORY INFORMATION** | | | | | | | | |
| Unique identifier | H306 (D23-0329) | H302 (D21-0956) | H281 | H297 | | | | |
| Donor age (years) | 46 | 33 | 56 | 60 | | | | |
| Donor sex (M/F) | F | F | F | M | | | | |
| Donor BMI (kg/m$^2$) | 25.5 | 46.4 | 27.34 | 25.1 | | | | |
| Donor $HbA_{1c}$ or other measure of blood glucose control | No insulin requirement, random BGL 8.9mmol/L | Not recorded | Not recorded | No insulin requirement, random BGL 8.6 | | | | |
| Origin/source of islets[b] | NSW | National Islet Transplant Consortium Australia | National Islet Transplant Consortium Australia | National Islet Transplant Consortium Australia | | | | |
| Islet isolation centre | Westmead Hospital | Westmead Hospital | Westmead Hospital | Westmead Hospital | | | | |
| Donor history of diabetes? Please select yes/no from drop down list | No | No | No | No | | | | |
| **If Yes, complete the next two lines if this information is available** | | | | | | | | |
| Diabetes duration (years) | | | | | | | | |
| Glucose-lowering therapy at time of death[c] | | | | | | | | |

| RECOMMENDED INFORMATION | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Donor cause of death | Intracranial hemorrhage | Cerebral ischemia | MVA – traumatic brain injury | Hemorrhagic cerebrovascular accident | | | | |
| Warm ischaemia time (h) | Not available | Not available | Not available | Not available | | | | |
| Cold ischaemia time (h) | 14.5 | 16.8 | 5.85 | 5.18 | | | | |
| Estimated purity (%) | 70% | 40% | 80% | 25% | | | | |
| Estimated viability (%) | 100% | 90% | 80% | 95% | | | | |
| Total culture time (h)[d] | <24 h | <24 h | <24 h | <24 h | | | | |
| Glucose-stimulated insulin secretion or other functional measurement[e] | Not performed | Not performed | Not performed | Not performed | | | | |
| Handpicked to purity? Please select yes/no from drop down list | No | No | No | No | | | | |
| Additional notes | Used in Figures 1C, 1D | Used in Figures 1C, 1D | Used in Figure 5F | Used in Figure 5F | | | | |