(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) **World Intellectual Property Organization**
International Bureau

(43) **International Publication Date**
23 May 2019 (23.05.2019)

WIPO | PCT

(10) **International Publication Number**
WO 2019/098944 A1

(51) **International Patent Classification:**
C12N 5/071 (2010.01) *A61P 3/10* (2006.01)
A61K 35/39 (2015.01)

(21) **International Application Number:** PCT/SG2018/050567

(22) **International Filing Date:** 14 November 2018 (14.11.2018)

(25) **Filing Language:** English

(26) **Publication Language:** English

(30) **Priority Data:**
62/586,323 15 November 2017 (15.11.2017) US

(71) **Applicant: NATIONAL UNIVERSITY OF SINGAPORE** [SG/SG]; 21 Lower Kent Ridge Road, Singapore 119077 (SG).

(72) **Inventors: TRYGGVASON, Karl**; c/o Duke-NUS Medical School, 8 College Road, Singapore 169857 (SG). **SIGMUNDSSON, Kristmundur**; c/o Duke-NUS Medical School, 8 College Road, Singapore 169857 (SG). **OJALA, Juha**; c/o Karolinska Institutet, Dept of Medical Biochemistry and Biophysics, 17177 Stockholm (SE).

(74) **Agent: SPRUSON & FERGUSON (ASIA) PTE LTD**; P.O. Box 1531, Robinson Road Post Office, Singapore 903031 (SG).

(81) **Designated States** *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

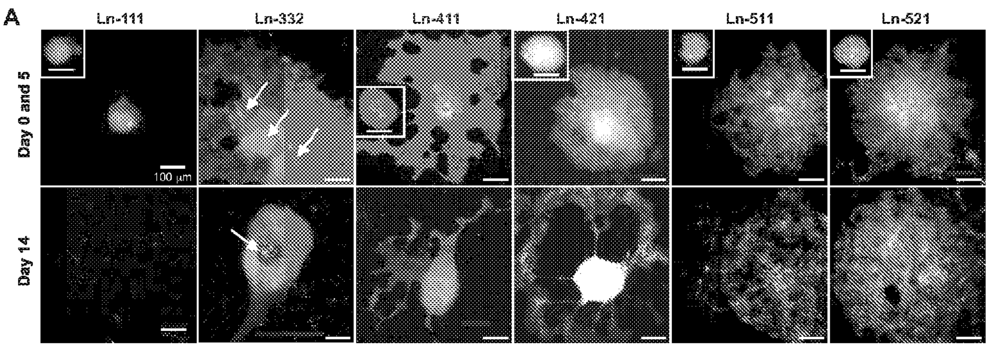
(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:
— *of inventorship (Rule 4.17(iv))*

Published:
— *with international search report (Art. 21(3))*
— *in black and white; the international application as filed contained color or greyscale and is available for download from PATENTSCOPE*

(54) **Title:** SYSTEMS FOR EXPANDING PANCREATIC ISLETS AND TRANSPLANTATION THEREOF

FIG. 1A



(57) **Abstract:** Methods of growing functional pancreatic islets in vitro and transplanting them into a mammalian species for treating metabolic disorders such as insulin deficiency are disclosed. Generally, islets are isolated, plated on a laminin matrix, and cultured in a cell culture medium to expand the pancreatic islets, wherein the laminin matrix contains laminin-521, laminin-51 1, laminin-421, laminin-41 1, or laminin-332, or a mixture thereof. Devices comprising a laminin matrix and pancreatic islets can be transplanted into a diabetic mammal, and are shown to regulate glucose levels.

WO 2019/098944 A1

SYSTEMS FOR EXPANDING PANCREATIC ISLETS AND TRANSPLANTATION THEREOF

[0001] This application claims priority to U.S. Provisional Patent Application Serial No. 62/586,323 filed November 15, 2017, and is incorporated in its entirety herein.

BACKGROUND

[0002] The present disclosure relates to methods for supporting long-term adhesion and expansion / proliferation of whole pancreatic islets containing one or more cell types, (i.e. alpha, beta, delta, gamma, or epsilon cells) that, respectively, produce glucagon, insulin, pancreatic peptide (PP), somatostatin, or ghrelin. Compositions and matrices for use in such methods are also disclosed, as well as devices that include such pancreatic islets. Transplantation of the expanded hormone producing pancreatic islets is further disclosed and included herein. These methods, compositions, devices, and systems comprising the same are useful for treating metabolic diseases such as diabetes.

[0003] Type 1 diabetes mellitus (T1D) is a metabolic disease in which the pancreas fails to produce enough insulin, resulting in high blood glucose levels over a prolonged period. Symptoms of high blood glucose levels include frequent urination, increased thirst and hunger, blurry vision, and fatigue. Type 2 diabetes is usually found in people aged 40 and above, but it can also develop in younger individuals who are overweight and physically inactive. In some people, the condition is mild and they are able to control their blood glucose with just diet and exercise. However, if the condition gets worse, oral medication or insulin injections may be required in addition to making lifestyle changes. Left untreated, diabetes can cause many complications, including heart disease, stroke, chronic kidney failure, foot ulcers, eye damage, and death. In some cases, Type 2 diabetes also benefits from treatment with exogenic insulin.

[0004] Physicians are constantly seeking new forms of diabetic treatment to either bolster the effects of or supplant current diabetic treatments such as drug therapy and organ transplantation. It would be desirable to identify novel effective methods of treating Type 1 and Type 2 diabetes.

BRIEF DESCRIPTION

[0005] Disclosed in various embodiments herein are methods and compositions (i.e. media, etc.) for expanding pancreatic islets using laminin matrices. It is contemplated that devices made from a laminin matrix and pancreatic islets can also be transplanted into mammals, such as humans, as a treatment for Type 1 diabetes or Type 2 diabetes. The expansion media are fully defined and xeno-free, enabling at least five weeks of *in vitro* expansion of whole functional islets on the laminin matrices. Longer culture and expansion periods are also possible. Murine and non-human primate islets have been shown to attach and expand on laminin matrices. Generally speaking, pancreatic islets are isolated and then expanded on the laminin matrix.

[0006] In one aspect, the present disclosure is directed to compositions, and methods of utilizing the same, to expand pancreatic islets. The islets are plated on a laminin matrix. The laminin matrix may contain a mixture of several laminins, or be formed from a single laminin. The laminin matrix may be affixed to a substrate if desired.

[0007] Also disclosed herein are methods for expanding pancreatic islets, comprising: plating pancreatic islets on a laminin matrix; and culturing the pancreatic islets in a cell culture medium to expand the pancreatic islets. In particular embodiments, the cell culture medium contains 15 mM or less of glucose and is free of Phenol red.

[0008] The laminin matrix may contain one of more of the following laminin isoforms: laminin-521, laminin-511, laminin-421, laminin-411, laminin-332, or laminin-111. In particular embodiments, the laminin matrix contains a single laminin isoform, and the single laminin is a complete laminin trimer (of alpha, beta and gamma chains) or a laminin chain, or a laminin fragment. The single laminin can be laminin-521, laminin-511, laminin-421, laminin-411, or laminin-332.

[0009] The culturing of the pancreatic islets may be performed in an atmosphere that is enriched in CO_2. For example, the culturing may occur in an atmosphere containing from about 5% CO_2 to about 10% CO_2.

[0010] In some embodiments, the cell culture medium is made from at least one RPMI medium and also contains 10 vol% serum, but in certain cases the serum may not be needed. The cell culture medium contains no unknown proteins, lipids, or growth factors; and contains glutathione, biotin, vitamin B12, PABA, inositol, and choline.

[0011] In other embodiments, the cell culture medium is mTeSR1 medium, or a similar cell culture medium.

[0012] The pancreatic islets may be cultured for at least one day. In more particular embodiments, the pancreatic islets are cultured for a period of about three (3) days to about 40 days, and can be cultured for longer than 40 days as well. The pancreatic islets may have a size of about 50 μm to over 500 μm.

[0013] Also contemplated are devices for treating Type 1 diabetes. Such a device comprises a laminin layer containing a laminin matrix, preferably coated upon a surface of a solid substrate; and a layer of pancreatic islets upon the laminin layer.

[0014] The laminin matrix contains laminin-521, laminin-511, laminin-421, laminin-411, laminin-332, or laminin-111. In particular embodiments, the laminin matrix contains a single laminin, and the single laminin is a complete laminin trimer, or is a laminin chain, or is a laminin fragment. The single laminin can be laminin-521, laminin-511, laminin-421, laminin-411, or laminin-332.

[0015] When present, the solid substrate is used to support the laminin layer, and can be made of polydimethylsiloxane (PDMS) or other similar biocompatible plastic materials, or made of biodegradable materials, as discussed further herein.

[0016] In specific embodiments, the solid substrate can be in the shape of a circular film, but other sizes or forms of solid substrates such as spheres can be used. The laminin layer does not have to coat the entire surface of the solid substrate. For instance, a perimeter that is laminin-free may be present. The layer of pancreatic islets may contain a varying amount of islets, and in some particular embodiments has a total of from about 100 to about 200 islets. These islets can vary in size, and can range from about 50 μm to about 500 μm, or greater.

[0017] In another aspect, the present disclosure is related to methods for treating a mammal with a metabolic disorder such as diabetes. The methods comprise transplanting the pancreatic islets described above into the mammal, sometimes along with the laminin matrix, or devices including the same.

[0018] The device can be implanted or grafted into a kidney of the mammal, or any other location that provides a vascular bed for the transplanted islets. The mammal may be a human. The pancreatic islets of the device can be human pancreatic islets, mouse pancreatic islets, monkey pancreatic islets, pig pancreatic islets, or rat pancreatic islets, or pancreatic islets from other mammals.

[0019] These and other non-limiting characteristics of the disclosure are more particularly disclosed below.

BRIEF DESCRIPTION OF THE DRAWINGS

[0020] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0021] **FIG. 1A** is a set of images arranged in two rows and six columns showing the adhesion, migration, and expansion of isolated mouse pancreatic islets plated onto plastic culture plates coated with, in order from left to right, laminin-111, laminin-332, laminin-411, laminin-421, laminin-511 or laminin-521. The top row is day 0 (top left insert) and day 5. The bottom row is day 14.

[0022] **FIG. 1B** is a set of six images comparing islets spreading for five days on LN-521 or EHS mouse-tumor-derived Matrigel in a 96-well microtiter plate. The top row is LN-521, and the bottom row is Matrigel. The leftmost column is day 1. The middle column is day 5, and the rightmost column is a magnified view of day 5.

[0023] **FIG. 1C** indicates the spreading efficiency in relation to initial islet size, applying the islets from **FIG. 1B**. The y-axis is area increase per islet, and runs from 0 to 15 in intervals of 5. The x-axis is the islet initial area, in μm^2, and is logarithmic. The x-axis indicates values from $5x10^3$ to $4x10^4$. Triangles are for LN-521, and circles are for Matrigel.

[0024] **FIG. 1D** shows islets from the same batch as in **FIG. 1B** after cultivation on LN-521 for three weeks. The top picture is day 1, and the bottom picture is day 18.

[0025] **FIG. 1E** illustrates that islets that were expanded for 18 days in culture show active synthesis of insulin and DNA. The images are arranged in two rows and four columns. The top row is anti-C-peptide antibody, and the bottom row is isotype control.

The far left column shows the culture. The center left column shows C-peptide/DAPI/EdU staining. The center right column shows DAPI/EdU staining. The right column shows EdU staining.

[0026] **FIG. 1F** is a quantification of the percentage of β-cells per islet and of proliferating β-cells from **FIG. 1E**. The left graph is % β-cells per islet, and the axis ranges from 0% to 100% in intervals of 20%. The right graph is % of proliferating β-cells, and the axis ranges from 0% to 20% in intervals of 5%.

[0027] **FIG. 1G** is an illustration of non-attached spherical islets from mTomato mouse that developed central hypoxia, which induced necrosis within 18 hours. The images are arranged in two rows and six columns. The top row is stained with Hoechst (blue), MAR (green), and DRAQ7 (red). The bottom row is stained with Hoechst (blue), mTomato (orange), and DRAQ7 (red). The columns are labeled, from left to right, 1 hour, 10 hours, 18 hours, 36 hours, 58 hours, and 70 hours.

[0028] **FIGS. 2A-2L** are a set of images showing the staining of islet cells, at day five of islet culturing on Laminin 521, addressing hormone production and quantification of islet composition thereof. **FIG. 2A** shows C-peptide staining of insulin producing β-cells, in red. **FIG. 2B** shows glucagon staining of α-cells, in blue. **FIG. 2C** shows somatostatin staining of δ-cells, in green.

[0029] **FIG. 2D** shows the merged view of C-peptide and somatostatin staining (**FIG. 2A** + **FIG. 2C**), indicating no overlapping in production of these hormones within islet cells.

[0030] **FIG. 2E** shows the merged view of pancreatic polypeptide (orange) and somatostatin staining (green = **FIG. 2C**), indicating no overlapping in production of these hormones within islet cells.

[0031] **FIG. 2F** shows a merged view of glucagon (blue = **FIG. 2B**) and pancreatic polypeptide (pp-peptide, the orange part of **FIG. 2E**), indicating a total overlap in expression of these hormones within α-cells.

[0032] **FIG. 2G** shows a merged view for the four hormones, indicating in addition to glucagon and pp-peptide, these α-cells also express insulin. **FIG. 2H** shows the same as **FIG. 2G**, with a nuclear DAPI (cyan) staining added to the merged view, providing the total amount of endocrine cells for this islet counting 400 cells. **FIG. 2I** shows the same as **FIG. 2H**, with the addition of membrane-Tomato to the merged view.

[0033] **FIG. 2J** is the merged view of **FIG. 2G** for the non-specific background control, where the four secondary antibodies were added to the islets in absence of the primary antibodies, keeping the same microscopy settings. Size bar = 50 μm.

[0034] **FIG. 2K** is the merged view of **FIG. 2H** for the non-specific background control, where the four secondary antibodies were added to the islets in absence of the primary antibodies, keeping the same microscopy settings. Size bar = 50 μm.

[0035] **FIG. 2L** is the merged view of **FIG. 2I** for the non-specific background control, where the four secondary antibodies were added to the islets in absence of the primary antibodies, keeping the same microscopy settings. Size bar = 50 μm.

[0036] **FIG 2M** provides the DAPI based cell counting for 23 islets that were analyzed with the Columbus software (PerkinElmer) applying a segmentation algorithm based on the immunofluorescence approach described above, which results are provided in **FIG 2N**, with average values and SEM provided as black crosses. Image data was collected on an Operetta high content screening microscope, applying a 20x NA objective. In **FIG. 2M**, the axis is labeled "endocrine cells per islet", and runs from 0 to 1200 in intervals of 200. In **FIG. 2N**, the axis is labeled "cell type per islet (%)", and runs from 0 to 100 in intervals of 20. Five sets of results are seen in **FIG. 2N**. The leftmost is for alpha cells, the center left is for beta cells, the center is for gamma cells, the center right is for PP-cells, and the right is triple positive.

[0037] **FIG. 3A** is a bar graph illustrating the gene expression levels (FPKM) of integrin genes in freshly isolated mouse islets (day 0). Previously reported laminin-binding integrins are highlighted in red (integrin alpha 6, Itga6; integrin alpha 3, Itga3 and integrin beta 4, Itgb4).

[0038] **FIG. 3B** is a bar graph indicating the number of differentially expressed genes after multiple testing correction with each laminin coating at day 12 compared to day 3 (Benjamini-Hochberg adjusted $P<0.05$).

[0039] **FIG. 3C** is a graphic expressing the top five functionally enriched KEGG pathways and GO terms when running Gene Set Enrichment Analysis (GSEA) ranking the mouse genome by differential expression at day 12 versus day 3 with LN-521, LN-421, and LN-111.

[0040] **FIG. 3D** is a graphic illustrating known connections among the LN-521 gene signature retrieved from the STRING database. The downregulated genes are Ero1lb, Uggt1, Magt1, Hspa5, Pyy, and Gfpt1. The genes identified as "focal adhesion" are Flnc, Flna, Mylk, Myl9, Ltga11, Thbs1, Spp1, and Fn1. All other genes are upregulated.

[0041] **FIG. 4A** shows two schematic descriptions. The top is a schematic description of how islets are cultured on LN-521-coated PDMS membranes. The bottom is a schematic description showing how the membranes are transplanted into the kidney capsule of a mouse.

[0042] **FIG. 4B** is a set of two pictures eight weeks after implantation. The top picture shows the implanted device on the mouse kidney capsule. The bottom picture shows the implanted device removed, and the original area where the device was implanted showing the red fluorescence of transplanted ActinDs-Red islets.

[0043] **FIG. 4C** is a set of images indicating that, 8 weeks after transplantation, the kidney is removed and ActinDs-Red positive transplanted islets produce C-peptide; i.e proving that the islets are functional and produce insulin. The islets are well vascularized by the endothelial cells derived from the host.

[0044] **FIG. 4D** is a set of two graphs indicating the glucose curves of Streptozotocin induced T1D mice before and after the transplantation of PDMS membranes with 110-130 islets growing on LN-521 into the kidneys thereof. For both graphs, the y-axis is glucose (mM) and is labeled from 5 to 35 in intervals of 10, and the x-axis is weeks before/after transplantation (transplantation=week 0). Streptozotocin was administered 2 weeks before transplantation, and kidneys were removed after 8 weeks. The top graph shows the results for each individual mouse. The bottom graph shows aggregate results for female mice receiving membranes cultured for 3 days, male mice receiving membranes cultured for 3 days, and male mice receiving membranes cultured for 7 days. **FIG. 4E** is a graph comparing male mice to female mice, and **FIG. 4F** is a graph aggregating all of the mice together.

[0045] **FIG. 5A** is a graph indicating the glucose curves of Streptozotocin induced T1D mice before and after the transplantation of 75 medium size (LM-M75 80 %, 100-

150 μm) and 32 large (LM-L32 30%, 150-250 μm) islets growing on LN-521 into the kidneys thereof after scraping the islets off the cell culture plastic with a cell scraper and settling the islets into sterile PE 50 polyethylene tubing (Intramedic) suitable for injection under the kidney capsule. The success rate of injection is estimated to be 80% for the medium-size islets and 30% for the large-size islets. The y-axis is glucose (mM) and is labeled from 0 to 35 in intervals of 5, and the x-axis is days before/after transplantation (transplantation=day 0). Streptozotocin was administered 28 days before transplantation, and kidneys were removed after 49 days. Small improvements of glucose levels are seen after the transplantation.

[0046] **FIG. 5B** is a graph indicating the glucose curves of Streptozotocin induced T1D mice before and after the transplantation of 150 small (S150 100%, 70-100 μm), 150 medium size (M150 90 %, 100-150 μm), or 64 large (L64 100%,150-250 μm) freshly isolated islets into the kidneys thereof after settling the islets into sterile PE 50 polyethylene tubing (Intramedic) suitable for injection under the kidney capsule. The success rate of injection is estimated to 100%, 90% and 100% for the small, medium, and large islets respectively. The y-axis is glucose (mM) and is labeled from 0 to 35 in intervals of 5, and the x-axis is days before/after transplantation (transplantation=day 0). Streptozotocin was administered 28 days before transplantation, and kidneys were removed after 49 days. Medium size islets resulted in normal blood glucose levels after 7 weeks while there was a small improvement, although not as good, with small and large size islets.

[0047] **FIG. 5C** is a graph indicating the glucose curves of Streptozotocin induced T1D mice before and after a sham transplantation operation of the kidneys (i.e. no islets were transplanted). The y-axis is glucose (mM) and is labeled from 0 to 35 in intervals of 5, and the x-axis is days before/after transplantation (transplantation=day 0). Streptozotocin was administered 28 days before transplantation, and kidneys were removed after 49 days. No improvement of blood glucose was observed, indicating that all the islets in the mice receiving transplants in **FIG. 5A** and **FIG. 5B** were rendered non-functional by streptozotocin and any improvement of blood glucose seen was due to the transplanted islets.

[0048] **FIG. 6** is a graph indicating the glucose curves of two Streptozotocin induced T1D mice before and after the transplantation of PDMS membranes with 140 islets growing on LN-521 under the skin over left shoulder blade thereof. The y-axis is glucose (mM) and is labeled from 0 to 35 in intervals of 5, and the x-axis is days before/after transplantation (transplantation=day 0). Streptozotocin was administered 7 days before transplantation, and the blood glucose was followed for 56 days. Both mice showed blood glucose improvement close to normal values 8 weeks after transplantation.

[0049] **FIGS. 7A-7G** are a set of images following a glucose-stimulated insulin secretion (GSIS) assay.

[0050] **FIG. 7A** is a graphic of a GSIS assay, indicating the ratio of glucose input to insulin output.

[0051] **FIG. 7B** is an image of a Western blot analysis of GSIS assay samples for large size (150 µm to 250 µm) islets cultured on LN-521 or uncoated plastic, and exposed to either 2 mM or 25 mM glucose.

[0052] **FIG. 7C** is a bar graph of a GSIS assay indicating insulin released (ng/mL) per large size islet cell (150 µm to 250 µm) cultured on LN-521 or LN-111 at day 3 and day 12, with the capacity to secrete insulin, stimulated by glucose levels of 25 mM per islet. The dark bar is LN-521, and the white bar is LN-111.

[0053] **FIG. 7D** is a bar graph of a GSIS assay indicating insulin released (ng/mL) per medium size islet cell (100 µm to 150 µm) cultured on LN-521 or LN-111 at day 3 and day 12. The dark bar is LN-521, and the white bar is LN-111.

[0054] **FIG. 7E** is a bar graph of a GSIS assay indicating insulin released (ng/mL) per small size islet cell (70 µm to 100 µm) cultured on LN-521 or LN-111 at day 3 and day 12, with the capacity to secrete insulin, stimulated by glucose levels of 25 mM per islet. The dark bar is LN-521, and the white bar is LN-111.

[0055] **FIG. 7F** is a bar graph of the ratio of insulin released upon exposure to 25 mM glucose to insulin released upon exposure to 2 mM glucose of large, medium, and small islets cultured on LN-521 or LN-111. The dark bar is LN-521, and the white bar is LN-111.

[0056] **FIG. 7G** is a bar graph of insulin released at 25 mM glucose, in picograms (pg), per beta cell of islet cells cultured on LN-521 or LN-111. The dark bar is LN-521, and the white bar is LN-111. Statistical significance for **FIGS. 7C-7G** is indicated by: (*) P<0.05, (**) P<0.01, and (***) P<0.001.

DETAILED DESCRIPTION

[0057] The present disclosure may be understood more readily by reference to the following detailed description of desired embodiments and the examples included therein. In the following specification and the claims that follow, reference will be made to a number of terms which will be defined to have the following meanings.

[0058] Although specific terms are used in the following description for the sake of clarity, these terms are intended to refer only to the particular structure of the embodiments selected for illustration in the drawings and are not intended to define or limit the scope of the disclosure. In the drawings and the following description below, it is to be understood that like numeric designations refer to components of like function. Furthermore, it should be understood that the drawings are not to scale.

[0059] The singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise.

[0060] As used in the specification and in the claims, the term "comprising" may include the embodiments "consisting of" and "consisting essentially of." The terms “comprise(s),” “include(s),” “having,” “has,” “can,” “contain(s),” and variants thereof, as used herein, are intended to be open-ended transitional phrases, terms, or words that require the presence of the named ingredients/steps and permit the presence of other ingredients/steps. However, such description should be construed as also describing compositions or processes as "consisting of" and "consisting essentially of" the enumerated ingredients/steps, which allows the presence of only the named ingredients/steps, along with any impurities that might result therefrom, and excludes other ingredients/steps.

[0061] Numerical values in the specification and claims of this application should be understood to include numerical values which are the same when reduced to the same number of significant figures and numerical values which differ from the stated value by less than the experimental error of conventional measurement technique of the type described in the present application to determine the value.

[0062] All ranges disclosed herein are inclusive of the recited endpoint and independently combinable (for example, the range of “from 2 grams to 10 grams” is inclusive of the endpoints, 2 grams and 10 grams, and all the intermediate values).

[0063] The term “about” can be used to include any numerical value that can vary without changing the basic function of that value. When used with a range, “about” also discloses the range defined by the absolute values of the two endpoints, e.g. “about 2 to about 4” also discloses the range “from 2 to 4.” The term “about” may refer to plus or minus 10% of the indicated number.

[0064] The present disclosure refers to “pancreatic islet cells” or “pancreatic islets.” These references should be understood as referring to the alpha, beta, gamma, delta, or epsilon cells in the pancreas that produce different hormones.

[0065] Over 320,000 patients in the United Kingdom have Type 1 diabetes (T1D), of which 5% experience more than seven hypoglycemic events per year (approximately 16,000 patients) and 10% have severely impaired hypoglycemic awareness (approximately 32,000 patients). Thus, it is clear that there is a high unmet medical need for development of improved therapeutic strategies for this patient population. Although technologies such as insulin pumps combined with continuous glucose monitoring (CGM) can be efficient for treatment of T1D and T2D, this is not a suitable option for all patients and results in no reduction of insulin dependency or improvement of hypoglycemic awareness.

[0066] The current cadaveric islet transplant approach can both reduce severe hypoglycemic events (SHEs) and improve hypoglycemic awareness. It is also much less invasive compared to whole pancreas transplantation. However, it is limited by the lack of available donor organs, with less than on average 30-40 transplantations per year in the United Kingdom. The current cost of the procedure, which typically requires transplantation from minimum two separate donors, is also prohibitively expensive.

[0067] With an increasing incidence rate of T1D, and also of insulin-dependent Type 2 diabetes (T2D) with high incidence of SHE and impaired awareness of hypoglycemia (IAH), the number of patients that could benefit from islet transplantation is set to increase substantially over time. Reducing SHE and IAH, and achieving insulin independence are desirable goals.

[0068] Recommendations from health economics analysts suggest that in the United Kingdom, a cell product that requires immunosuppression is unlikely to be adopted by the National Health Service (NHS) for a patient population of more than 400 patients due to limited cost-benefit of such treatment. However, the same analysis demonstrates that the treatment methods disclosed in the present disclosure could become cost-effective following a minimum reduction of 7 SHE per year even on immunosuppressive treatment. Furthermore, if a reduction of more than 20 SHE per year can be reached, the treatment is cost-effective at the same price as of a single transplantation using the current human islet approach. According to Collaborative Islet Transplant Registry (CITR) records, patients that have so far received human islet transplantation decreased the average number of SHE per year from 23 to as few as 2. Although it is not clear how many patients have more than 20 SHE per year, it is likely to be significantly more than the patients currently on the transplant list for either pancreas and/or human islet transplantation. This suggests that there may be a sizeable market for pancreatic islet transplantation treatments (such as those described in the present disclosure). In the U.S.A. and the U.K., there is a total diabetes population of over 32 million, with a T1D population of almost 1.8 million.

[0069] Developing improved therapeutic solutions for T1D and T2D is a major focus for both multinational pharmaceutical corporations as well as emerging biotechnology and biomedical companies. These efforts are focused on the following areas: long-acting insulin derivatives and insulin biosimilars; therapeutics to increase insulin production; advanced insulin pumps combined with continuous glucose monitoring; autoimmune and immunomodulatory therapies; transplantation strategies including generation of insulin producing cells, such as xenogenic islets, human stem cell and iPSC approaches; encapsulation technologies for enhancement of transplantation strategies; generation of artificial pancreas, including repopulation of decellularized pancreas and 3D organ bioprinting; and therapeutics for stimulation of regeneration of beta cells in situ.

[0070] There are currently over 180 therapies in clinical development (Phase I-III) for treatment of T1D and/or T2D, including therapies to counteract or prevent secondary complications such as neuropathy and nephropathy. The main competing technology to transplantation is the development of more sophisticated insulin pumps in conjunction with continuous glucose monitoring (CGM). The two main advantages of insulin pumps compared to transplantation are the lower cost of treatment and that there is no need of immunosuppression. However, currently insulin pumps are not suitable for all patients, and such systems do not provide the additional functionality of transplanted islets.

[0071] It is contemplated that whole differentiated pancreatic islets can be grown, expanded and further transplanted into human subjects with the methods of the present disclosure. Generally speaking, the present disclosure provides a laminin matrix, upon which pancreatic islets are plated and cultured. This can take the form of a substrate support that is coated with specific basement membrane laminins. The pancreatic islets cultured and expanded on this laminin matrix, once transplanted into a patient, provide sufficient normalization of blood glucose levels. It is also possible to transplant only the islets themselves (i.e. without the laminin matrix). A xeno-free and chemically defined cell culture medium system is used with the laminin matrix to expand the pancreatic islets, including all possible cell types.

[0072] A laminin protein comprises one α-chain subunit, one β-chain subunit, and one γ-chain subunit, all joined together in a trimer through a coiled-coil domain. The twelve known laminin subunit chains can form at least 16 trimeric laminin types in native mammalian tissues. Within the trimeric laminin structures are identifiable domains that possess binding activity towards other laminin and basal lamina molecules, as well as cell plasma membrane-bound receptors. For example, domains VI, IVb, and IVa form globular structures, and domains V, IIIb, and IIIa (which contain cysteine-rich EGF-like elements) for rod-like structures. Domains I and II of the three chains participate in the formation of a triple-stranded coiled-coil structure (the long arm). There exist five genetically different alpha chains, three beta chains, and three gamma chains that have been found in human tissues in at least sixteen different combinations. One nomenclature for these laminin proteins describes the isoforms based on their chain compositions, e.g., laminin-111 (laminin-1) that contains alpha-1, beta-1, and gamma-1 chains.

[0073] The term "laminin-521" refers to the protein isoform formed by joining α5, β2, and γ1 chains together. The term "laminin-511" refers to the protein formed by joining α5, β1, and γ1 chains together. The term "laminin-421" refers to the protein formed by joining α4, β2, and γ1 chains together. The term “laminin-411” refers to the protein formed by joining α4, β1, and γ1 chains together. The term “laminin-332” refers to the protein formed by joining α3, β3, and γ2 chains together. These terms should be construed as encompassing both the recombinant laminin and heterotrimeric laminin from naturally occurring sources. The term “recombinant” indicates that the protein is artificially produced in cells that do not normally express such proteins. These terms may also be used to refer to the parts of such protein isoforms.

[0074] The laminin can be a complete protein trimer, or a protein chain, or a protein fragment. The term “complete” refers to the protein being composed of all the domains of the α-chain, one β-chain, and γ-chain, with the three chains being joined together to form the heterotrimeric structure. The protein is not broken down into separate chains, fragments, or functional domains. The term “chain” refers to the entirety of the individual alpha, beta, or gamma chain of the laminin protein. The term “fragment” refers to any protein fragment that contains one, two, or three functional domains that possesses binding activity to another molecule or receptor. However, a chain should not be considered a fragment because each chain possesses more than three such domains. Similarly, a complete laminin protein trimer should not be considered a fragment. Examples of functional domains include Domains I, II, III, IV, V, VI, and the G domain.

[0075] The present disclosure relates to methods for culturing and expanding pancreatic islet cells, i.e. to obtain a greater number of islet cells from a smaller number of cells, also known as proliferation. Very generally, the pancreatic islets are cultured on a laminin matrix which can be affixed to a solid substrate, which acts as a support. The term “solid” refers to the state of matter, i.e. the substrate is not liquid or gas or plasma. The substrate can be rigid / hard or very flexible (such as a film), and the term “solid” should not be construed as requiring a particular degree of rigidity.

[0076] The laminin matrix may contain a mixture of multiple laminins, or may contain a single laminin trimer, or a single laminin chain, or a single laminin fragment. The term “single” is used herein to mean that the laminin matrix contains only one laminin trimer or chain or fragment, but permits other ingredients to still be present in the laminin matrix. The pancreatic islets are then nourished using a cell culture medium that contains a small amount of glucose, fifteen (15) millimolar (mM) or less. The cell culture medium also does not contain Phenol red, which is also known as phenolsulfonphthalein.

[0077] Primary cells typically require two things to survive and reproduce outside of their natural environment. First, a structural support for the cell is needed. Second, a cell culture medium provides nutrition to the cell. In the present disclosure, the structural support for the cells is a laminin matrix. The pancreatic islets are then deposited upon the laminin matrix. As desired, the laminin matrix can be formed upon a substrate, which can be a rigid or flexible material that provides a surface upon which the laminin matrix is formed. The substrate can be, for example, a petri dish or the well of a multi-well plate. In various transplantable devices described herein, the substrate can also be a thin film or membrane made of a biocompatible polymer like polydimethylsiloxane (PDMS), polyvinyl chloride (PVC), polytetrafluoroethylene (PTFE), polyethersulfone (PES), polyethylene (PE), polyetheretherketone (PEEK), polysulfone (PS), polypropylene (PP), polyethylene glycol (PEG), polyvinyl alcohol (PVOH), polymethyl methacrylate (PMMA), polyethylene vinyl acetate (EVA), poly(ether urethane), polyethylene terephthalate, polyethylene oxide, polyethylene oxide-co-polypropylene oxide, or polyacrylamide. The substrate could alternatively be made from a biodegradable material like polylactic acid, polyglycolic acid, poly(ε-caprolactone), poly(dioxanone), poly(lactide-co-glycolide), polyglyconate, or polyorthoester, or a hydrogel.

[0078] As mentioned above, the laminin matrix on the substrate can contain any effective laminin. It is specifically contemplated that the laminin matrix contains only one particular laminin (i.e. one single laminin), though other ingredients may also be present in the laminin matrix. In particular embodiments, the laminin is Laminin-511 (LN-511), Laminin-521 (LN-521), Laminin-411 (LN-411), Laminin-421 (LN-421), or Laminin-332 (LN-332). It is particularly contemplated that the laminin matrix does not contain a cadherin. Cadherins are also known as desmogleins and desmocollins, and are absent from the laminin matrix.

[0079] The laminin matrix is used in combination with a cell culture medium. Typically, cell culture media include a large number and a large amount of various growth factors and cytokines to inhibit differentiation and improve proliferation.

[0080] Of relevance to the cell culture medium for the pancreatic islet cells of the present disclosure is the physiological range for glucose concentration, which can range from 3.9 mM to 7.7 mM in plasma, depending on fasting conditions and time measured after eating. Glucose concentration in serum can range from 3.9 mM to 11.0 mM. By nature, the beta cells of the pancreas are exceptionally sensitive to glucose, and can become stressed after long-term exposure to glucose levels that are above physiological concentrations, which in serum is about 3.9 to 11 mM. An additional concern from the physiological point of view, regarding effects on cell differentiation and gene regulation (and for high quality fluorescence microscopy), is the presence of Phenol Red indicator in the cell culture medium. For these reasons, the cell culture media used in the present methods have a glucose level that is within physiological glucose concentrations and are free of Phenol Red. In particular embodiments, the cell culture media have a glucose level of 15 mM or lower, including 11 mM or lower, and including 5 mM or lower. However, media containing higher concentrations of glucose may also be used.

[0081] RPMI 1640 medium was originally developed to culture human leukemic cells in suspension and as a monolayer. Roswell Park Memorial Institute (RPMI) 1640 medium is unique from other media because it contains the reducing agent glutathione and high concentrations of vitamins. RPMI 1640 medium contains biotin, vitamin B12, and PABA, which are not found in Eagles Minimal Essential Medium or Dulbecco's Modified Eagle Medium. In addition, the vitamins inositol and choline are present in very high concentrations in RPMI 1640. RPMI 1640 medium contains no proteins, lipids or growth factors. Most commercially available RPMI 1640 media have 2 g/L glucose (= 11.1 mM), which is at the upper borders of the average physiological level.

[0082] In some embodiments, the cell culture medium of the present disclosure is obtained by mixing at least one RPMI 1640 medium together with serum. More specifically, the following reagents, available from GIBCO and listing the GIBCO catalog number, are used:

Ingredients	**GIBCO Catalog No.**
RPMI 1640 – free of Phenol Red; 11.1 mM Glucose	11835030 or 11835055

SILAC RPMI 1640 Flex Media – free of Phenol Red, D-Glucose, L-Arginine, L-Lysine	A2494201
GlutaMax (100x) (200 mM L-alanyl-L-glutamine in 0.85% NaCl)	35050-061
Penicillin-Streptomycin (100x) (10,000 U/mL penicillin, 10,000 µg/mL streptomycin)	15140122
Fetal bovine serum – heat inactivate before use	26140087

[0083] For a final volume of 100 mL, the two RPMI 1640 media are mixed together in a 1:1 volume ratio to make 90 mL to decrease the glucose concentration. 10 mL of FBS is then added to obtain a serum amount of 10% of final volume, resulting in 10% FBS/RPMI 1640 medium. Because of practicality, the Glutamax (1:100) and penicillin-streptomycin (1:100) are then added at volumes of 1 mL each, resulting in a final medium which has a glucose concentration of 4.90 mM (= ((11.1 mM / 2)* 90/102). This cell culture medium is designed to meet the needs of pancreatic islets.

[0084] As mentioned above, the final glucose concentration of the cell culture medium should be in desired embodiments 15 mM or less, and in particular embodiments be 11 mM or less, or even 5 mM or less. Preferably, the glucose concentration is at least 3.5 mM, which is just under the low end of physiological concentrations listed above.

[0085] RPMI 1640 medium requires supplementation, commonly with 10% FBS, because no proteins, lipids, or growth factors are present. The serum may be from about 8 vol% to about 12 vol% of the cell culture medium. RPMI 1640 medium uses a sodium bicarbonate buffer system (2.0 g/L), and therefore requires a 5-10% CO_2 environment to maintain physiological pH. In other embodiments, however, it is contemplated that serum is not needed.

[0086] Alternatively, another cell culture medium that can be used contains RPMI 1640 medium and a Connaught Medical Research Laboratories (CMRL) medium mixed together in a 1:1 ratio and supplemented with fetal calf serum to obtain a serum amount of 10% of the final volume, and containing 10 mM HEPES, 1% GlutaMax, 0.5% penicillin, and 0.5% streptomycin. This cell culture medium has a glucose concentration of ~7.43 mM.

[0087] It is contemplated that the cell culture medium will be completely defined and xeno-free. Therefore, desirably it should not contain sera that are usually from animals (e.g. bovine serum) that can vary from batch to batch. The medium should also be devoid of any differentiation inhibitors, feeder cells, or differentiation inductors, or apoptosis inhibitors. Examples of feeder cells include mouse fibroblasts or human dermal fibroblasts. Examples of differentiation inductors include Noggin or keratinocyte growth factor.

[0088] Alternatively, the cell culture medium may be mTeSR1 cell culture medium, which can contain up to 4-6 mM glucose. The ingredients of this cell culture medium are listed in the following Table 1, though it is contemplated that the amount of each individual ingredient can vary up to 5% in each direction:

Table 1. mTeSR1 formulation.

mTeSR1 Ingredient	**molar mass (g/mol)**	**Concentration (ng/mL)**	**Concentration (mM)**
INORGANIC SALTS			
Calcium chloride (Anhydrous)	110.98	9.14E+04	8.24E-01
HEPES	238.3	2.81E+06	1.18E+01
Lithium Chloride (LiCl)	42.39	4.15E+04	9.80E-01
Magnesium chloride (Anhydrous)	95.21	2.26E+04	2.37E-01
Magnesium Sulfate ($MgSO_4$)	120.37	3.84E+04	3.19E-01
Potassium chloride (KCl)	74.55	2.43E+05	3.26E+00
Sodium bicarbonate ($NaHCO_3$)	84.01	1.51E+06	1.80E+01
Sodium chloride (NaCl)	58.44	5.53E+06	9.46E+01
Sodium phosphate, dibasic (Anhydrous)	141.96	5.56E+04	3.92E-01
Sodium phosphate, monobasic monohydrate (NaH_2PO_4-H_2O)	137.99	4.90E+04	3.55E-01
TRACE MINERALS			
Ferric Nitrate ($Fe(NO_3)_3$-$9H_2O$)	404	3.92E+01	9.71E-05
Ferrous sulfate heptahydrate ($FeSO_4$-$7H_2O$)	278.01	3.28E+02	1.18E-03
Copper(II) sulfate pentahydrate ($CuSO_4$-$5H_2O$)	249.69	1.02E+00	4.08E-06
Zinc sulfate heptahydrate ($ZnSO_4$-$7H_2O$)	287.56	3.39E+02	1.18E-03
Ammonium Metavanadate NH_4VO_3	116.98	1.28E+00	1.09E-05
Manganese Sulfate monohydrate ($MnSO_4$-H_2O)	169.02	3.33E-01	1.97E-06
$NiSO_4$-$6H_2O$	262.85	2.55E-01	9.70E-07
Selenium	78.96	1.40E+01	1.77E-04
Sodium Meta Silicate Na_2SiO_3 $9H_2O$	284.2	2.75E+02	9.66E-04

mTeSR1 Ingredient	molar mass (g/mol)	Concentration (ng/mL)	Concentration (mM)
$SnCl_2$	189.62	2.35E-01	1.24E-06
Molybdic Acid, Ammonium salt	1235.86	2.43E+00	1.97E-06
$CdCl_2$	183.32	2.24E+00	1.22E-05
$CrCl_3$	158.36	3.14E-01	1.98E-06
$AgNO_3$	169.87	1.67E-01	9.81E-07
$AlCl_3$ $6H_2O$	241.43	1.18E+00	4.87E-06
Barium Acetate ($Ba(C_2H_3O_2)_2$)	255.42	2.50E+00	9.79E-06
$CoCl_2$ $6H_2O$	237.93	2.33E+00	9.81E-06
GeO_2	104.64	5.20E-01	4.97E-06
KBr	119	1.18E-01	9.89E-07
KI	166	1.66E-01	1.00E-06
NaF	41.99	4.13E+00	9.83E-05
RbCl	120.92	1.19E+00	9.81E-06
$ZrOCl_2$ $8H_2O$	178.13	1.75E+00	9.80E-06
ENERGY SUBSTRATES			
D-Glucose	180.16	2.47E+06	1.37E+01
Sodium Pyruvate	110.04	4.31E+04	3.92E-01
LIPIDS			
Linoleic Acid	280.45	5.27E+01	1.88E-04
Lipoic Acid	206.33	8.25E+01	4.00E-04
Arachidonic Acid	304.47	3.93E+00	1.29E-05
Cholesterol	386.65	4.33E+02	1.12E-03
DL-alpha tocopherol-acetate	472.74	1.37E+02	2.90E-04
Linolenic Acid	278.43	1.95E+01	6.99E-05
Myristic Acid	228.37	1.96E+01	8.59E-05
Oleic Acid	282.46	1.96E+01	6.94E-05
Palmitic Acid	256.42	1.96E+01	7.65E-05
Palmitoleic acid	254.408	1.96E+01	7.71E-05
Stearic Acid	284.48	1.96E+01	6.89E-05
AMINO ACIDS			
L-Alanine	89.09	1.22E+04	1.37E-01
L-Arginine hydrochloride	147.2	8.07E+04	5.48E-01
L-Asparagine-H_2O	150.13	2.06E+04	1.37E-01
L-Aspartic acid	133.1	1.82E+04	1.37E-01
L-Cysteine-HCl-H_2O	175.63	1.38E+04	7.83E-02

mTeSR1 Ingredient	molar mass (g/mol)	Concentration (ng/mL)	Concentration (mM)
L-Cystine dihydrochloride	313.22	2.45E+04	7.83E-02
L-Glutamic acid	147.13	2.02E+04	1.37E-01
L-Glutamine	146.15	4.30E+05	2.94E+00
Glycine	75.07	2.21E+04	2.94E-01
L-Histidine monohydrochloride monohydrate	209.63	2.47E+04	1.18E-01
L-Isoleucine	131.17	4.28E+04	3.26E-01
L-Leucine	131.17	4.64E+04	3.54E-01
L-Lysine hydrochloride	182.65	7.14E+04	3.91E-01
L-Methionine	149.21	1.35E+04	9.06E-02
L-Phenylalanine	165.19	2.79E+04	1.69E-01
L-Proline	115.13	2.49E+04	2.16E-01
L-Serine	105.09	3.09E+04	2.94E-01
L-Threonine	119.12	4.19E+04	3.52E-01
L-Tryptophan	204.23	7.07E+03	3.46E-02
L-Tyrosine disodium salt hydrate	225.15	3.78E+04	1.68E-01
L-Valine	117.15	4.16E+04	3.55E-01
VITAMINS			
Ascorbic acid	176.12	4.46E+04	2.53E-01
Biotin	244.31	2.74E+00	1.12E-05
B12	1355.37	5.34E+02	3.94E-04
Choline chloride	139.62	7.02E+03	5.03E-02
D-Calcium pantothenate	238.27	8.79E+02	3.69E-03
Folic acid	441.4	2.08E+03	4.71E-03
i-Inositol	180.16	9.89E+03	5.49E-02
Niacinamide	122.12	1.59E+03	1.30E-02
Pyridoxine hydrochloride	205.64	1.57E+03	7.62E-03
Riboflavin	376.36	1.72E+02	4.56E-04
Thiamine hydrochloride	337.27	8.16E+03	2.42E-02
GROWTH FACTORS/PROTEINS			
GABA	103.12	1.01E+05	9.79E-01
Pipecolic Acid	129	1.27E+02	9.84E-04
bFGF	18000	1.04E+02	5.77E-06
TGF beta 1	25000	5.88E-01	2.35E-08
Human Insulin	5808	2.28E+04	3.92E-03
Human Holo-Transferrin	78500	1.08E+04	1.37E-04
Human Serum Albumin	67000	1.31E+07	1.95E-01

mTeSR1 Ingredient	molar mass (g/mol)	Concentration (ng/mL)	Concentration (mM)
Glutathione (reduced)	307.32	1.96E+03	6.38E-03
OTHER COMPONENTS			
Hypoxanthine Na	136.11	1.61E+03	1.18E-02
Phenol red	354.38	5.99E+03	1.69E-02
Putrescine-2HCl	161.07	6.36E+01	3.95E-04
Thymidine	242.229	2.86E+02	1.18E-03
2-mercaptoethanol	78.13	7.66E+03	9.80E-02
Pluronic F-68	8400	1.96E+05	2.33E-02
Tween 80	1310	4.31E+02	3.29E-04

[0089] The source of the pancreatic islets can be any animal. However, in particular embodiments, the source is a mammal, such as a human, mouse, monkey, pig, or rat. After being isolated from their source, the pancreatic islets are plated on the laminin matrix, which is itself usually coated upon the substrate. The pancreatic islets are then cultured in the cell culture medium and expanded, typically for a period of at least 3 days and lasting as long as 35 days in culture, or 40 days in culture, or even longer. As mentioned above, this may be done in an atmosphere containing about 5% to about 10% CO_2 when the RPMI-based cell culture medium is used. As is well known, the culturing also occurs at elevated temperatures within ± 5°C of normal body temperature (37°C). Cell culture medium can be exchanged as desired. The expansion of islets occurs without removing them from the substrate and without dispersing the cells. Under those conditions all islet cell types proliferate at a similar rate. It is also possible to disperse the islets into single cells, then replate them and grow, but it is not necessary for expanding the islet cells (and results in unnecessary cell losses).

[0090] The resulting expanded pancreatic islets have normoglycemic function, i.e. they produce insulin in lesser or greater quantities depending on the amount of glucose in their environment. The pancreatic islets may have a size ranging from about 50 µm to about 500 µm, as measured by their diameter. Some human islets are even larger.

[0091] Also contemplated are medical devices that can be used to treat Type 1 diabetes or Type 2 diabetes. These devices can comprise a laminin layer containing a laminin matrix; and a layer of pancreatic islets upon the laminin layer. In some particular embodiments, the laminin layer is formed upon a solid substrate. If desired, and depending on the shape of the substrate, there can be two laminin layers on the substrate, one layer on each of the opposite surfaces of the substrate, and two layers of pancreatic islets (one layer of pancreatic islets on each of the laminin layers). It is contemplated here that the substrate is in the shape of a thin film or membrane, or is in the shape of a sphere or a fiber. A sphere or fiber would be considered to have only one surface, whereas a thin film or membrane would be considered to have two opposite surfaces. The substrate can be coated with the laminin matrix and used as surface(s) for adhesion and expansion of islets in vitro. Suitable materials for the membrane include polydimethylsiloxane (PDMS) and other biologically compatible substrates or biodegradable substrates as described above. The laminin layer does not have to cover the entire surface of the flexible substrate, as will be further explained in the Examples. Depending on the size of the substrate, the layer of pancreatic islets can contain a total of about 50 to several hundreds of whole islets, and even up to 1000 islets. In some more specific embodiments, the layer of pancreatic islets can contain from about 50 to about 1000 islets, or from about 50 to about 200 islets. Alternatively, in embodiments without a substrate, two layers of pancreatic islets could be placed on opposite surfaces of the laminin layer.

[0092] It is contemplated that these devices can be transplanted into a mammal, such as a human, who is suffering from diabetes (Type 1 or Type 2). The device can be implanted into any suitable location or organ or tissue with sufficient vascular bed, for example the pancreas, the kidney, muscle or subcutaneous tissue.

[0093] The present disclosure is further illustrated in the following non-limiting working examples, it being understood that these examples are intended to be illustrative only and that the disclosure is not intended to be limited to the materials, conditions, process parameters and the like recited herein.

EXAMPLES

EXAMPLE 1

[0094] Pancreatic islets were isolated from mice and monkeys, and plated onto wells containing 5 µg/cm^2 of one of six laminins (LN-111, LN-332, LN-411, LN-421, LN-511, LN-521) or Matrigel (an EHS mouse sarcoma tissue extract that contains several basement membrane components). mTeSR1 cell culture medium was used. **FIG. 1A** is a set of images showing the adhesion, migration, and expansion of isolated mouse pancreatic islets. Morphology of islets plated right after plating is provided in the top-left insert, except for islets plated on LN-332, where zero-hour islets are provided as overlay in green. The islets did not attach to LN-111. Islets plated on LN-511 and LN-521 attached and effectively spread in a chemically defined serum-free medium, LN-521 providing more rapid attachment and expansion than LN-511. The islets unwrapped from spheres into 2-3 cell layer sheets in just a few days while maintaining cell-cell contacts. Islets attaching to LN-332, LN-411 and LN-421 supported adhesion at day 5, but the spreading was not extensive and the islets shrank and developed gaps by day 14. At day 14, islets on LN-332, LN-411, and LN-421 undertook re-clustering (and central necrosis). Red coloring indicates DRAQ7 that binds to DNA in leaky and dying cells.

[0095] **FIG. 1B** illustrates the comparison of islets spreading for five days on LN-521 to Matrigel in a 96-well microtiter plate. For each condition, a typical islet is indicated with an arrow and provided at high magnification. Nuclei are stained with DAPI (blue) and β-cells spotted with anti-C peptide antibody (red). The islets on Matrigel did not expand, whereas those on LN-521 did. **FIG. 1C** indicates the spreading efficiency in relation to initial islet size for the islets from **FIG. 1B**. **FIG. 1D** shows islets from the same batch as in **FIG. 1B** after cultivation on LN-521 for three weeks.

[0096] In order to explore the proliferation rate of individual cell types in islets cultured on LN-521 for 18 days, the islets were treated with EdU and stained. **FIG. 1E** illustrates the islets expanded for 18 days in culture, and shows active synthesis of insulin (green coloring indicates C-peptide staining) and DNA (red coloring indicates EdU incorporation to nuclei). **FIG. 1F** is a quantification of the relative amount of β-cells per islet and of proliferating β-cells from **FIG. 1E**, provided 66.6 ± 6.3% and 11.2 ± 2.6%, respectively (average ± sem, n = 10). **FIG. 1G** is an illustration of non-attached spherical islets from mTomato mouse (orange cell membranes) that developed central hypoxia (top row, green: MAR), which induced necrosis within 18 hours (red: DRAQ7). Leaky and dead cells are indicated by blue Hoescht staining of nuclei. Non-attached whole islets rapidly expel the necrotic cells as clumps, indicating that a large proportion of β-cells are disabled early. In contrast, β-cells, mainly located on or close to the islet surface, survive better.

[0097] Another important observation was that after 2-3 weeks in culture on LN-521 the islet cells started to proliferate robustly. During the first two weeks in culture, proliferation of the islet cells was only about 0.5 to 2 %, but after 2-3 weeks all islet cell types (alpha, beta, gamma, delta) proliferated approximately 10% as determined by EdU staining.

EXAMPLE 2

[0098] Pancreatic islets were isolated from mice and plated onto wells containing LN-521 and allowed to spread for 5 days, followed by staining of 4 endocrine hormones, to verify the existence and specific synthesis activity of defined endocrine cell-types. mTeSR1 cell culture medium was used. **FIGS. 2A-2N** are images showing the staining of islet cells, at day five of islet culturing on Laminin 521, addressing hormone production and quantification of islet composition thereof. **FIG. 2A** shows C-peptide staining of insulin producing β-cells, in red. **FIG. 2B** shows glucagon staining of α-cells, in blue. **FIG. 2C** shows somatostatin staining of δ-cells, in green. **FIG. 2D** shows the merged view of C-peptide and somatostatin staining (A+C), indicating no overlapping in production of these hormones within islet cells. **FIG. 2E** shows the merged view of pancreatic polypeptide (orange) and somatostatin staining (green = C), indicating no overlapping in production of these hormones within islet cells. **FIG. 2F** shows a merged view of glucagon (blue = B) and pancreatic polypeptide (pp-peptide, the orange part of E), indicating a total overlap in expression of these hormones within α-cells. **FIG. 2G** shows a merged view for the four hormones, indicating in addition to glucagon and pp-peptide, these α-cells also express insulin. **FIG. 2H** shows the same as G, with a nuclear DAPI (cyan) staining added to the merged view, providing the total amount of endocrine cells for this islet counting 400 cells. **FIG. 2I** shows the same as H, with the addition of membrane-Tomato to the merged view. **FIG. 2J**, **FIG. 2K**, and **FIG. 2L** provide the merged views (as for **FIG. 2G**, **FIG. 2H**, and **FIG. 2I**, respectively) for the non-specific background control, where the four secondary antibodies were added to the islets in absence of the primary antibodies, keeping the same microscopy settings. Size bar = 50 μm. **FIG 2M** provides the DAPI based cell counting for 23 islets that got analized with the Columbus software (PerkinElmer) applying a segmentation algorithm based on the immunofluorescence approach described above, which results are provided in **FIG 2N**, with average values and SEM provided as black crosses. Image data was collected on a Operetta high content screening microscope, applying a 20x NA objective.

EXAMPLE 3

[0099] **FIG. 3A** reveals that integrin beta-1 (Itgb1) has the greatest basal gene expression levels (FPKM) in freshly isolated mouse islets (day 0) followed by integrin alphaV (Itgav) and integrin alpha-6 (Itga6). Previously reported laminin-binding integrins are highlighted in red (integrin alpha 6, Itga6; integrin alpha 3, Itga3 and integrin beta 4, Itgb4).

[0100] RNA sequencing was performed in order to look at differential gene expression on day 3 and day 12 in islets cultured on LN-521. **FIG. 3B** indicates the number of differentially expressed genes after multiple testing correction with each laminin coating at day 12 compared to day 3 (Benjamini-Hochberg adjusted $P<0.05$). LN-521 demonstrated large numbers of upregulated and downregulated differentially expressed genes, while LN-421 showed extremely limited upregulated but fairly moderate downregulated numbers of differentially expressed genes. LN-111 also showed an extremely limited upregulated number of differentially expressed genes but demonstrated an extremely high number of downregulated differentially expressed genes.

[0101] LN-521 resulted in the up-regulation of several genes enriched for KEGG pathways and molecular processes related to adhesion and cell-to-cell interaction, including “focal adhesion”, “vascular smooth muscle contraction”, “regulation of cytoskeleton” and “ECM-receptor interactions” pathways. In contrast, coating with LN-111 or LN-421 resulted in down-regulation (or no significant change) of these processes and pathways. For, example, at day 12, when the islets had transformed into flat 1-3 cell layer islets, integrin $\alpha 11$ was upregulated four-fold in islets on a LN-521 coating (Benjamini Hochberg (BH) adjusted P-value = 1.2×10^{-8}). However, the $\alpha 11$ integrin expression did not change in islets growing on LN-111 or LN-421 coatings. Integrin $\alpha 11$ as well as other integrins are reported to bind collagens I, IV and IX, and other proteins such as fibronectin or VCAM.

[0102] The top five functionally enriched KEGG pathways and GO terms are graphed in **FIG. 3C**. These were determined by running Gene Set Enrichment Analysis (GSEA) ranking the mouse genome by differential expression at day 12 versus day 3 with LN-521, LN-421, and LN-111. In the level graph, color is mapped to GSEA normalized enrichment score (NES) of -5 to 5. The higher the NES value, the stronger the enrichment for the pathway that contains the genes that are downregulated. Results are displayed as sorted by LN-521 NES values. Significant results after multiple testing correction are denoted by ** (GSEA FDR < 0.05). Nominally significant results are denoted by * (GSEA P < 0.05). Lack of any * notation denotes non-significant results (GSEA P > 0.05). Islets cultured on LN-521 indicated strong enrichment in the KEGG pathway and GO BP and GO MF terms but weaker enrichment in the nuclear part and organelle part of the GO CC term. These results are directly opposite to those seen in islets cultured on either LN-421 and LN-111, which showed weaker enrichment in the KEGG pathway and GO BP and GO MF terms but stronger enrichment in the nuclear part and organelle part of the GO CC term.

[0103] Known connections among the LN-521 gene signature were retrieved from the STRING database and mapped. In the network graph of **FIG. 3D**, each node represents a gene from the LN-521 gene signature, and each edge (connection) represents an interaction between a pair of genes as reported in the STRING database. STRING protein-protein interactions are highlighted in red. Node size is mapped to the absolute Log2 gene expression fold change in day 12 compared to day 3. Up regulated genes (positive Log2 fold change) at day 12 compared to day 3 with LN-521 coating are colored in red, whereas down regulated genes (negative Log2 fold chance) at day 12 compared to day 3 with LN-521 coating are colored in blue. Genes annotated as part of the KEGG pathway “focal adhesion” (the top enriched KEGG pathway in the LN-521 gene signature) are highlighted with a black node border. The graph contains only the genes that form a network out of the 104 genes that are included in the LN-521 gene signature.

EXAMPLE 4

[0104] In addition to RNA sequencing, pancreatic islets were grown on laminin coated films and transplanted under the kidney capsule of streptozotocin induced T1D mice. A schematic description of islets cultured on LN-521 coated PDMS membranes is shown at the top of **FIG. 4A**. A PDMS membrane (blue) is placed in a 6-well plate overlaid with casts with 5 and 3 mm diameter openings. The inner 3 mm cast is used for laminin coating (yellow), and it is removed after overnight adhesion of the plated islets (black dots). The outer cast is used to facilitate the punching of the membrane into 5 mm diameter membranes used for the transplantation. 50-70 islets isolated from Actin-DsRed transgenic mice or normal C57Bl/6N mice were plated onto PDMS films coated overnight with LN-521, and then cultured for 3 days or 7 days prior to transplantation. The islets attached, flattened out, and expanded on the PDMS film.

[0105] The bottom of **FIG. 4A** is a schematic description of the transplantation procedure under the kidney capsule. A small incision is made to the dorsal left flank of the mouse under anesthesia. Through the opening, the left kidney is exposed and a needle is used to make an opening through the kidney capsule. Two PDMS membranes with cultured islets exposed towards kidney parenchyma are placed under the capsule on both sides of the kidney. The kidney is carefully pushed back into the peritoneum and the opening through the peritoneal wall, and the skin is sealed with sutures.

[0106] Following transplantation, blood glucose levels were evaluated at day 1 to 3 and later twice a week in the transplanted mice.

[0107] Eight weeks after transplantation, the kidneys were removed, dissected, and observed for vascularization. This is seen in **FIG. 4B**. The top picture indicates where the PDMS membrane was placed. The transplanted islets originated from Actin-DsRed mice showing red fluorescence that can be seen attached to the kidney parenchyme in the lower panel after the PDMS membrane is removed. This indicates that the membrane can be removed if desired without removing the transplanted islets.

[0108] **FIG. 4C** is a set of stains indicating the presence of transplanted islets originating from Actin-DsRed mice, C-peptide, and CD31 separately, then merged into one, and a flattened CD31 stain (showing a Z-stack of many layers combined). The yellow dotted line indicates the boundary between the transplanted islets isolated from Actin-DsRed mice (red cells, top right) and the kidney cortex tissue of a mouse to which the expanded islets were transplanted. Together, these stains show that the transplanted islets (red, top right) readily grew in vivo, produced insulin (C-peptide is a product of cleaved insulin) and that they were vascularized by host tissue blood vessels (non-red cells, bottom left).

[0109] **FIG. 4D** indicates the glucose curves of streptozotocin induced T1D mice before and after transplantation of PDMS membranes with 110-130 mouse islets grown on LN-521 for 3 or 7 days. Streptozotocin was administered 2 weeks before transplantation. A spike in blood glucose levels is seen, indicating death of the natural beta cells in the pancreas. Regardless of the sex of the animals, within 2-3 weeks, the blood glucose decreased from >28 mM down to normal values (< 11.1 mM) where it stayed until the transplant was removed. This indicated that the transplanted islets were producing insulin and responding physiologically to feeding. The islet transplant receiving kidneys were removed after 8 weeks, and the glucose levels spiked back up. This indicates efficacy of the transplanted islets in regulating glucose levels, i.e. normalization of hyperglycemia. **FIG. 4E** is a graph comparing male mice to female mice, and **FIG. 4F** is a graph aggregating all of the mice together.

EXAMPLE 5

[0110] Islets were cultured on a LN-521-coated substrate. Two mice then received islets that were scraped off the LN-521 coating (i.e. only the expanded islets were transplanted into the mice). One mouse received 75 islets (LM-M75), and the other mouse received 32 islets (LM-L32). **FIG. 5A** is a graph showing the glucose levels (mM) over time (days). The success of transplantation was estimated to be 80% and

30%, respectively. Prior to the transplantation the blood glucose was stably over 33.3 mM for one week and therefore any improvement in the blood glucose values is expected to come from the insulin produced by the small number of transplanted islets.

[0111] **FIG. 5B** is a graph showing the glucose levels (mM) over time (days) for round islets freshly isolated prior to transplantation in a separate experiment. One mouse received 64 large islets (150-250 μm, L64), one mouse received 150 medium islets (100-150 μm, M150), and one mouse received 150 small islets(70-100 μm, S150). The success of transplantation was estimated to be 100%, 90%, and 100% respectively. Medium size islets seemed to work better than small islets in improving the blood glucose level. Due to lower number of large islets, the comparison is not as clear. The normalization of blood glucose requires at least 7 weeks or higher number of transplanted islets when using freshly isolated islets.

[0112] **FIG. 5C** is a graph showing the glucose levels over time for three "dummy" mice that were operated upon, but received no islets (i.e. a sham operation). Prior to the transplantation the blood glucose was high, but still fluctuating below 33.3 mM, therefore any improvement in the blood glucose values after the sham operation is expected to follow the same pattern confirming that no real improvement of blood glucose occurred during the 7 week follow up time.

EXAMPLE 6

[0113] Islets were cultured on a LN-521-coated PDMS membrane for 3 days. Two streptozotocin treated mice with T1D then received 140 islets on the PDMS membrane that were transplanted subcutaneously on the left scapula. **FIG. 6** is a graph showing the glucose levels (mM) over time (days). Normoglycemia limit 11.1 mM is marked "LIMIT". Both mice show improvement in the blood glucose values close to normal levels at week 8 post-transplantation. The subcutaneous model of laminin-cultured islets has therefore similar efficacy as transplantation of round freshly isolated islets depicted in **FIG. 5B**, indicating that the flat islets withstand well the relatively hypoxic environment provided by the subcutaneous vascular bed in comparison to the kidney parenchyma.

EXAMPLE 7

[0114] Islets were cultured at 8 mM glucose levels then exposed to 2 mM for 1 hour. After collecting a sample, islets were then exposed to 25 mM glucose for 1 hour, after which another sample was collected and the culture continued. As shown in **FIG. 7A**, insulin output increased proportionally to glucose input, with increased beta cell efficacy at 25 mM glucose levels compared to 2 mM glucose levels.

[0115] A Western blot analysis of the GSIS assay was conducted for samples of large size islets (150-250 µm) cultured on either LN-521 or uncoated plastic. As shown in **FIG. 7B**, insulin output was observed in large size islets after exposure to 25 mM glucose and cultured on LN-521. However, no significant output (i.e. no visible insulin blot) was observed in islets cultured on LN-521 with 2 mM glucose or in islets cultured on uncoated plastic (not shown).

[0116] A GSIS assay was performed on large size islets (150-250 µm) cultured on either LN-521 or LN-111. Islets were observed for their capacities to secrete insulin stiumulated by glucose levels of 25 mM per islet. A graph comparing these capacities is shown in **FIG. 7C**, which indicates that islets cultured on LN-521 released more insulin than islets cultured on LN-111. From day 3 of culture to day 12 of culture, the amount of insulin released from islets cultured on LN-521 increased by a third, while the amount of insulin released from islets cultured on LN-111 remained the same.

[0117] A GSIS assay was performed on medium size islets (100-150 µm) cultured on either LN-521 or LN-111. Islets were observed for their capacities to secrete insulin stiumulated by glucose levels of 25 mM per islet. A graph comparing these capacities is shown in **FIG. 7D**, which indicates that islets cultured on LN-521 released more insulin than islets cultured on LN-111. From day 3 of culture to day 12 of culture, the amount of insulin released from islets cultured on LN-521 doubled, while the amount of insulin released from islets cultured on LN-111 minimally increased.

[0118] A GSIS assay was performed on small size islets (70-100 µm) cultured on either LN-521 or LN-111. Islets were observed for their capacities to secrete insulin stimulated by glucose levels of 25 mM per islet. A graph comparing these capacities is shown in **FIG. 7E**, which indicates that islets cultured on LN-521 released more insulin than islets cultured on LN-111. From day 3 of culture to day 12 of culture, the amount of insulin released from islets cultured on LN-521 and LN-111 remained approximately the same.

[0119] **FIG. 7F** is a graph comparing the results from the GSIS assays. Overall, islets cultured on LN-521 secreted more insulin than islets cultured on LN-111. Further, the larger the islet, the greater the amount of secreted insulin. Therefore, the greatest insulin secretion was observed in large islets cultured on LN-521. A broad comparison may be found in **FIG. 7G.**

[0120] Statistical significance for **FIGS. 7C-7G** is indicated by: (*) $P<0.05$, (**) $P<0.01$, and (***) $P<0.001$.

[0121] While particular embodiments have been described, alternatives, modifications, variations, improvements, and substantial equivalents that are or may be presently unforeseen may arise to applicants or others skilled in the art. Accordingly, the appended claims as filed and as they may be amended are intended to embrace all such alternatives, modifications, variations, improvements, and substantial equivalents.

CLAIMS:

1. A method for expanding pancreatic islets, comprising:
plating pancreatic islets on a laminin matrix; and
culturing the pancreatic islets in a cell culture medium to expand the pancreatic islets.

2. The method of claim 1, wherein the laminin matrix contains laminin-521, laminin-511, laminin-421, laminin-411, or laminin-332, or a mixture thereof.

3. The method of claim 1, wherein the laminin matrix contains a single laminin, and the single laminin is a complete laminin trimer, or a laminin chain, or a laminin fragment.

4. The method of claim 3, wherein the single laminin is laminin-521, laminin-511, laminin-421, laminin-411, or laminin-332.

5. The method of any one of claims 1-4, wherein the culturing of the pancreatic islets is performed in an atmosphere enriched in CO_2.

6. The method of any one of claims 1-5, wherein the cell culture medium is made from at least one RPMI medium.

7. The method of any one of claims 1-5, wherein the cell culture medium is mTeSR1 medium or similar without addition of serum.

8. The method of any one of claims 1-7, wherein the pancreatic islets are cultured for at least 1 day.

9. The method of any one of claims 1-8, wherein the pancreatic islets have a size of from about 50 μm to about 500 μm.

10. The method of any one of claims 1-9, wherein the laminin matrix is coated upon a substrate.

11. The method of any one of claims 1-10, wherein the cell culture medium contains 15 mM or less of glucose.

12. The method of claim 11, wherein the cell culture medium is free of Phenol Red.

13. A device for treating diabetes, comprising:
a laminin layer containing a laminin matrix; and
a layer of pancreatic islets upon the laminin layer.

14. The device of claim 13, wherein the laminin matrix contains laminin-521, laminin-511, laminin-421, laminin-411, or laminin-332, or a mixture thereof.

15. The device of claim 13, wherein the laminin matrix contains a single laminin, and the single laminin is a complete laminin trimer, or a laminin chain, or a laminin fragment.

16. The device of claim 15, wherein the single laminin is laminin-521, laminin-511, laminin-421, laminin-411, or laminin-332.

17. The device of any one of claims 13-16, wherein the layer of pancreatic islets contains from about 50 to about 1000 islets.

18. The device of any one of claims 13-17, wherein the device further comprises a solid substrate with a surface upon which the laminin layer is placed.

19. The device of claim 18, wherein the solid substrate is made of a biocompatible polymer.

20. The device of claim 18, wherein the solid substrate is made of a biodegradable material.

21. The device of any one of claims 18-20, wherein the solid substrate is in the shape of a circle or a sphere or a fiber.

22. The device of any one of claims 18-21, wherein the laminin layer coats the entire surface of the solid substrate or coats only part of the surface of the solid substrate.

23. A method for treating a mammal with diabetes, comprising transplanting the device of any one of claims 13-22 into the mammal.

24. The method of claim 23, wherein the device is implanted in a kidney of the mammal.

25. The method of claim 23, wherein the device is implanted in an organ or tissue where the islets of the device can become vascularized.

26. The method of any one of claims 23-25, wherein the mammal is a human.

27. The method of any one of claims 23-26, wherein the pancreatic islets of the device are human pancreatic islets, mouse pancreatic islets, monkey pancreatic islets, pig pancreatic islets, or rat pancreatic islets.

28. A method for treating a mammal with diabetes, comprising:
plating pancreatic islets on a laminin matrix; and
culturing the pancreatic islets in a cell culture medium; and
transplanting the cultured expanded pancreatic islets into an organ or tissue of the mammal that is capable of vascularizing the transplanted pancreatic islets.

29. A method for treating a mammal with diabetes, comprising:
plating pancreatic islets on a laminin matrix; and
transplanting the pancreatic islets on the laminin matrix into an organ or tissue of the mammal that is capable of vascularizing the transplanted pancreatic islets.

30. The method of claim 29, wherein the pancreatic islets are cultured and expanded on the laminin matrix in a cell culture medium prior to transplanting.

FIG. 1A

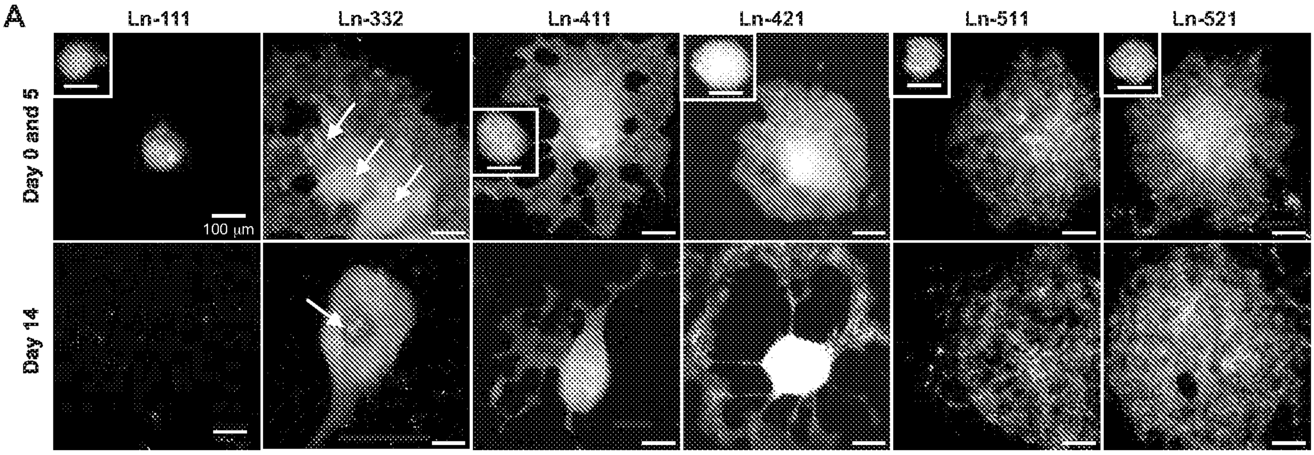
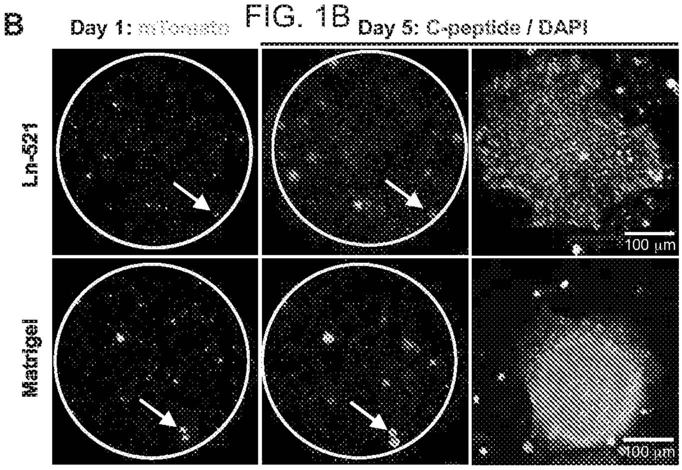


FIG. 1B



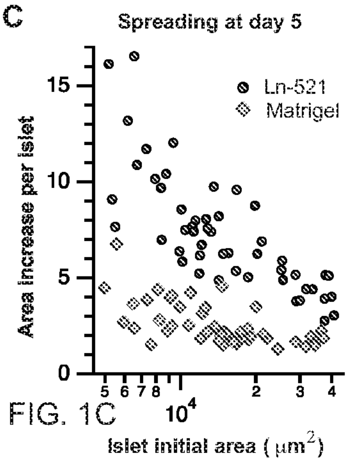


FIG. 1C

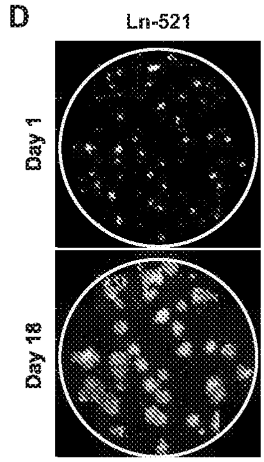
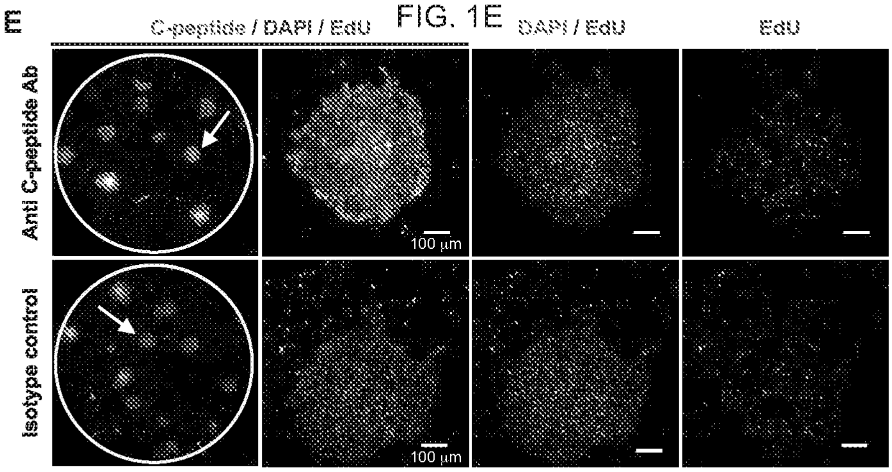


FIG. 1D

FIG. 1E



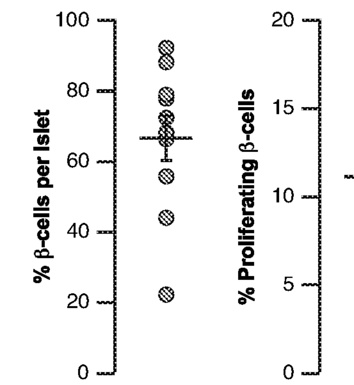


FIG. 1F

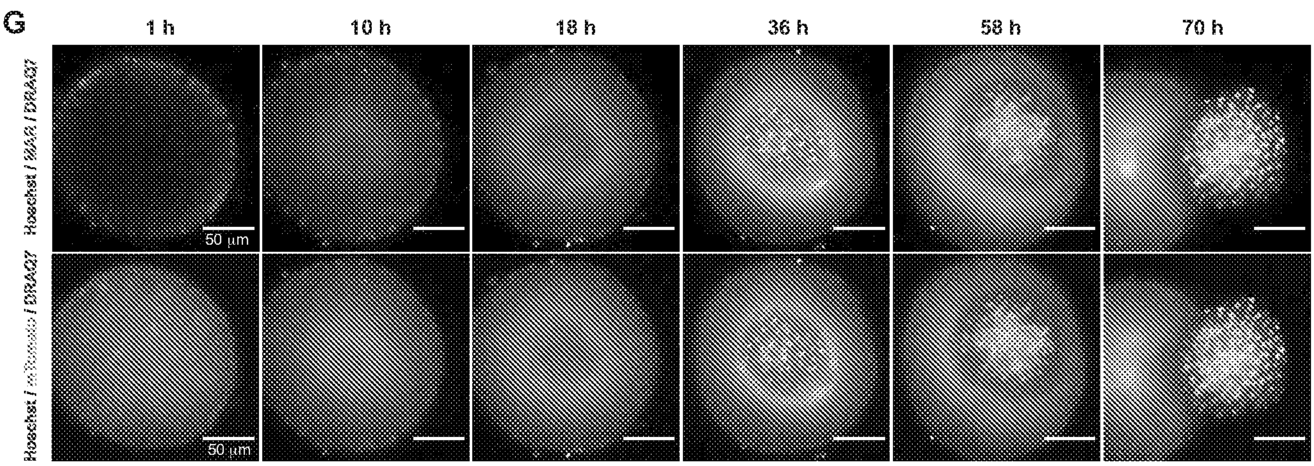


FIG. 1G

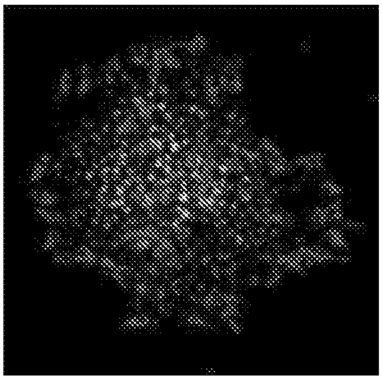


FIG. 2A

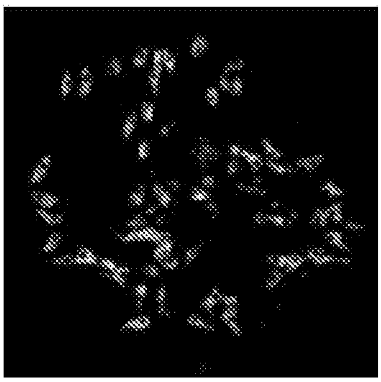


FIG. 2B

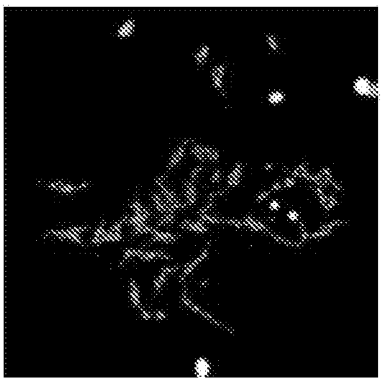


FIG. 2C

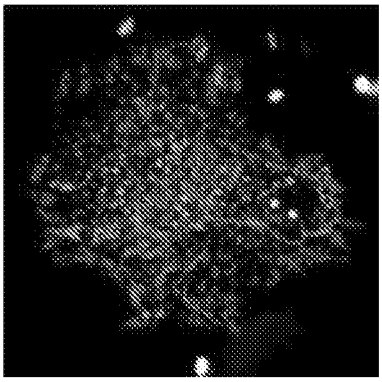


FIG. 2D

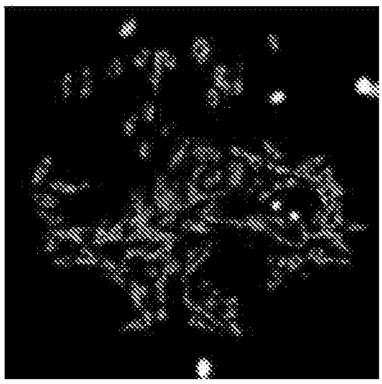


FIG. 2E

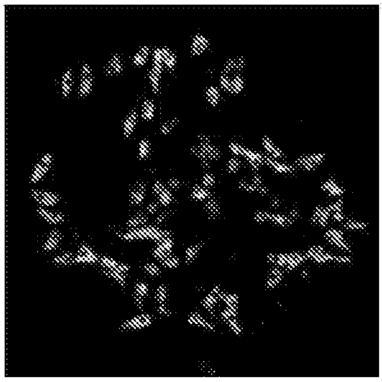


FIG. 2F

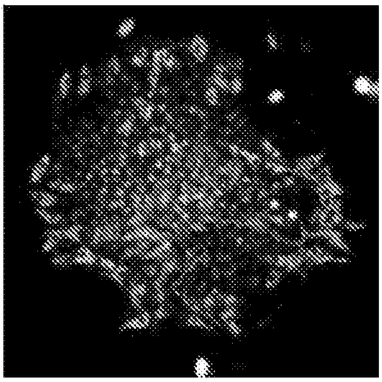


FIG. 2G

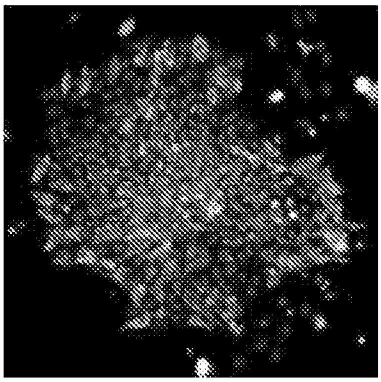


FIG. 2H

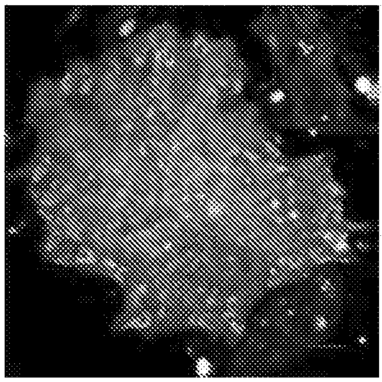


FIG. 2I

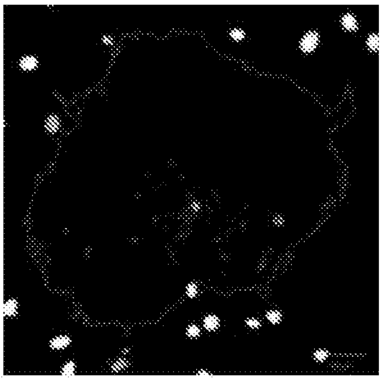


FIG. 2J

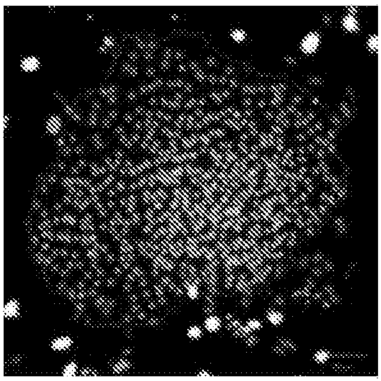


FIG. 2K

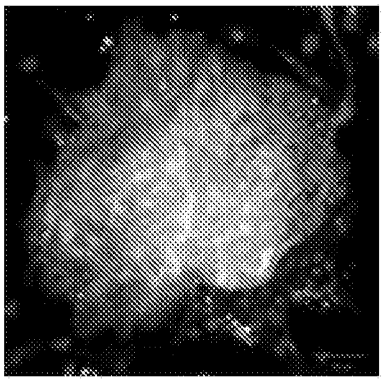


FIG. 2L

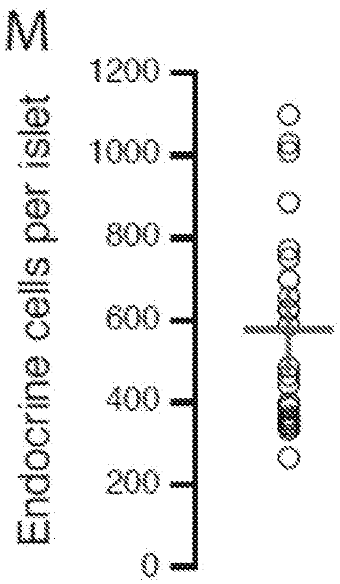


FIG. 2M

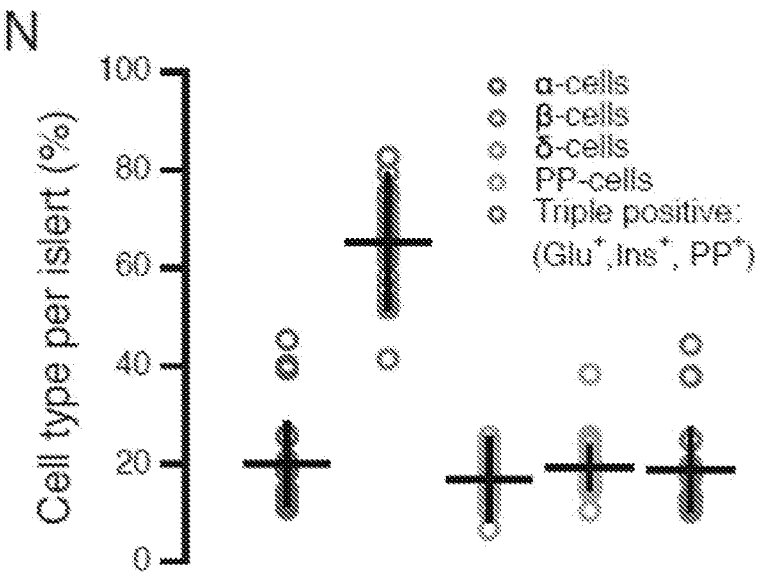


FIG. 2N

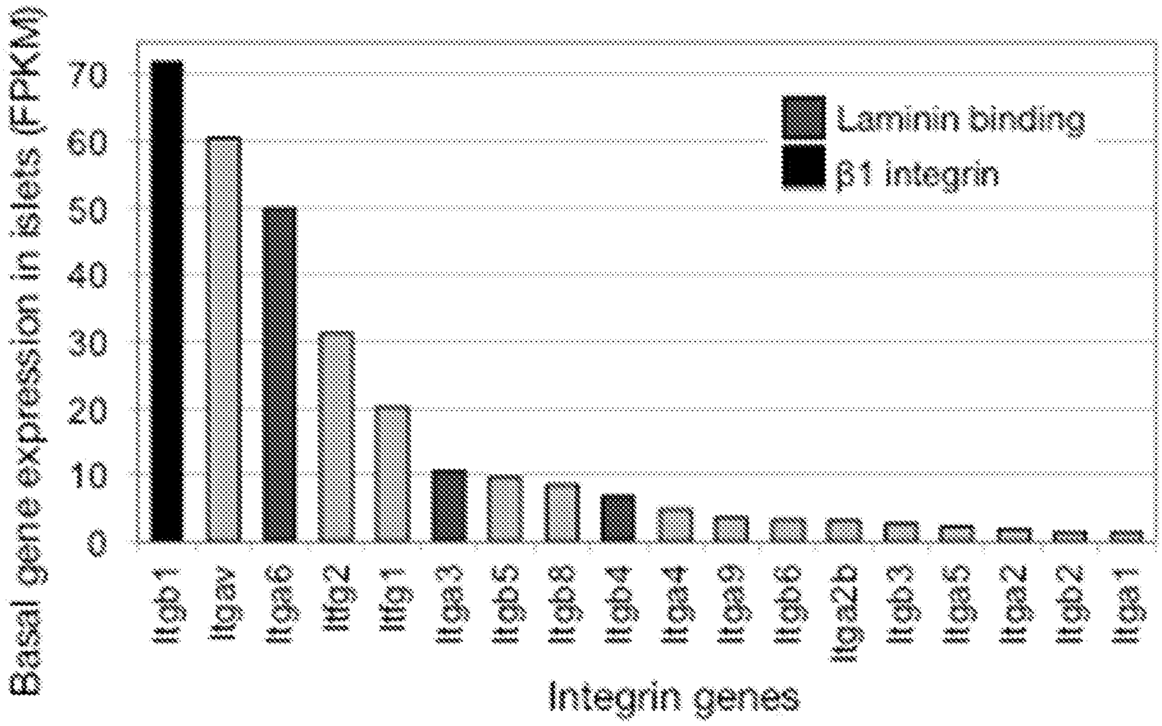

Basal gene expression in islets (FPKM)
70
60
50
40
30
20
10
0
Laminin binding
β1 integrin
Itgb1
Itgav
Itga6
Itfg2
Itfg1
Itga3
Itgb5
Itgb8
Itgb4
Itga4
Itga9
Itgb6
Itga2b
Itgb3
Itga5
Itga2
Itgb2
Itga1
Integrin genes

FIG. 3A

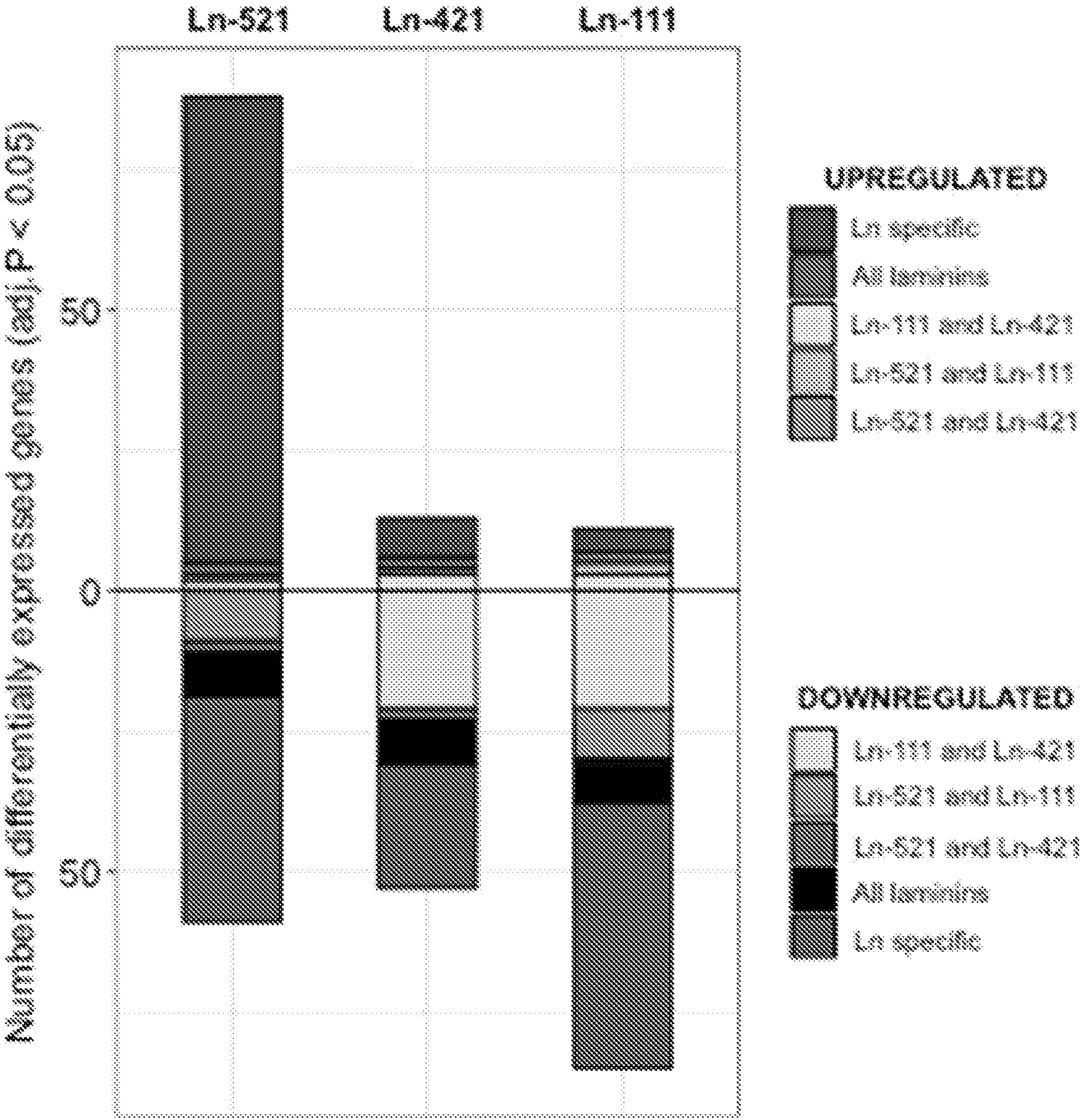

Ln-521
Ln-421
Ln-111
Number of differentially expressed genes (adj. P < 0.05)
50
0
50
UPREGULATED
Ln specific
All laminins
Ln-111 and Ln-421
Ln-521 and Ln-111
Ln-521 and Ln-421
DOWNREGULATED
Ln-111 and Ln-421
Ln-521 and Ln-111
Ln-521 and Ln-421
All laminins
Ln specific

FIG. 3B

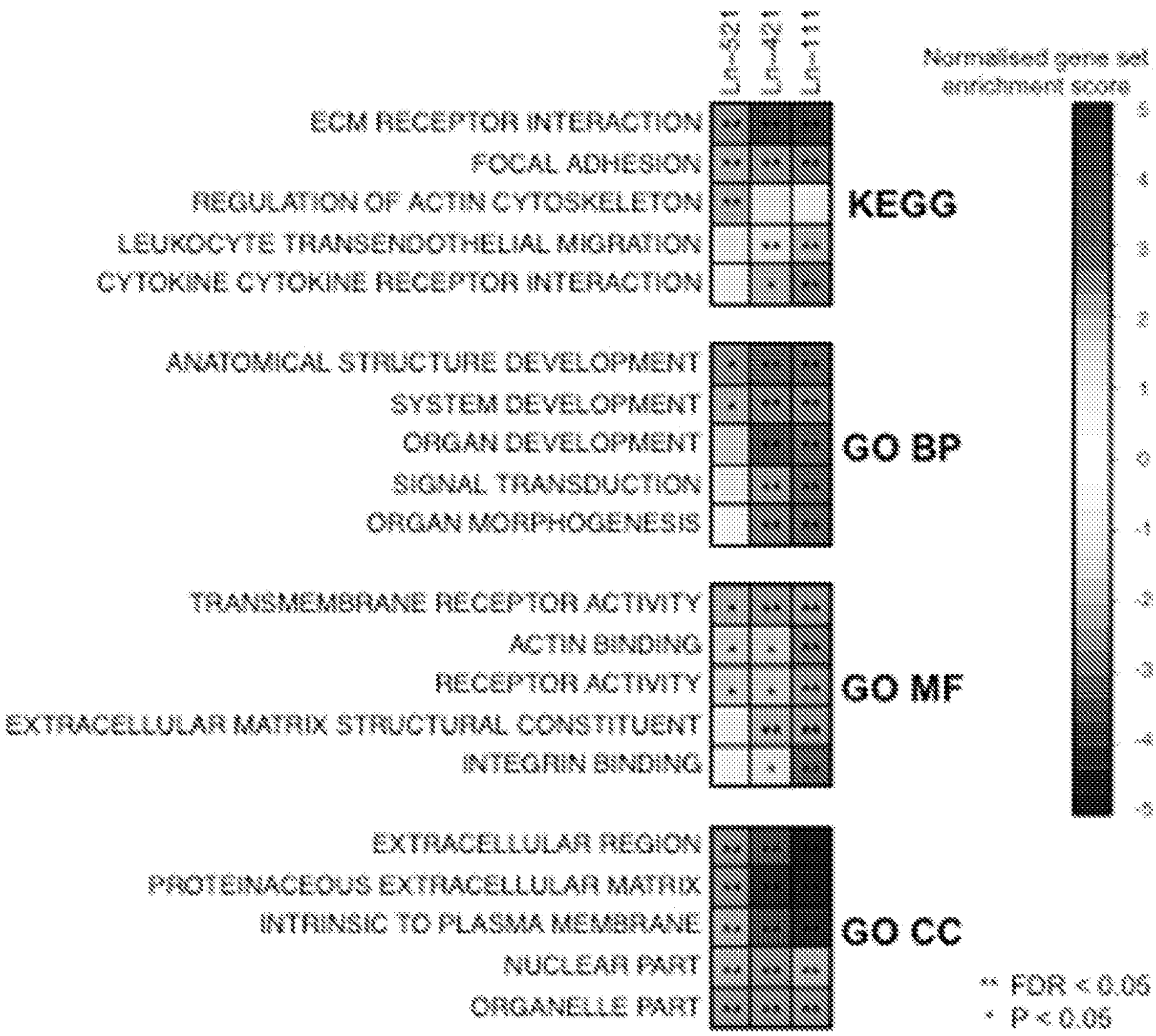

Normalised gene set enrichment score
5
4
3
2
1
0
-1
-2
-3
-4
-5
ECM RECEPTOR INTERACTION
FOCAL ADHESION
REGULATION OF ACTIN CYTOSKELETON
LEUKOCYTE TRANSENDOTHELIAL MIGRATION
CYTOKINE CYTOKINE RECEPTOR INTERACTION
KEGG
ANATOMICAL STRUCTURE DEVELOPMENT
SYSTEM DEVELOPMENT
ORGAN DEVELOPMENT
SIGNAL TRANSDUCTION
ORGAN MORPHOGENESIS
GO BP
TRANSMEMBRANE RECEPTOR ACTIVITY
ACTIN BINDING
RECEPTOR ACTIVITY
EXTRACELLULAR MATRIX STRUCTURAL CONSTITUENT
INTEGRIN BINDING
GO MF
EXTRACELLULAR REGION
PROTEINACEOUS EXTRACELLULAR MATRIX
INTRINSIC TO PLASMA MEMBRANE
NUCLEAR PART
ORGANELLE PART
GO CC
** FDR < 0.05
* P < 0.05

FIG. 3C

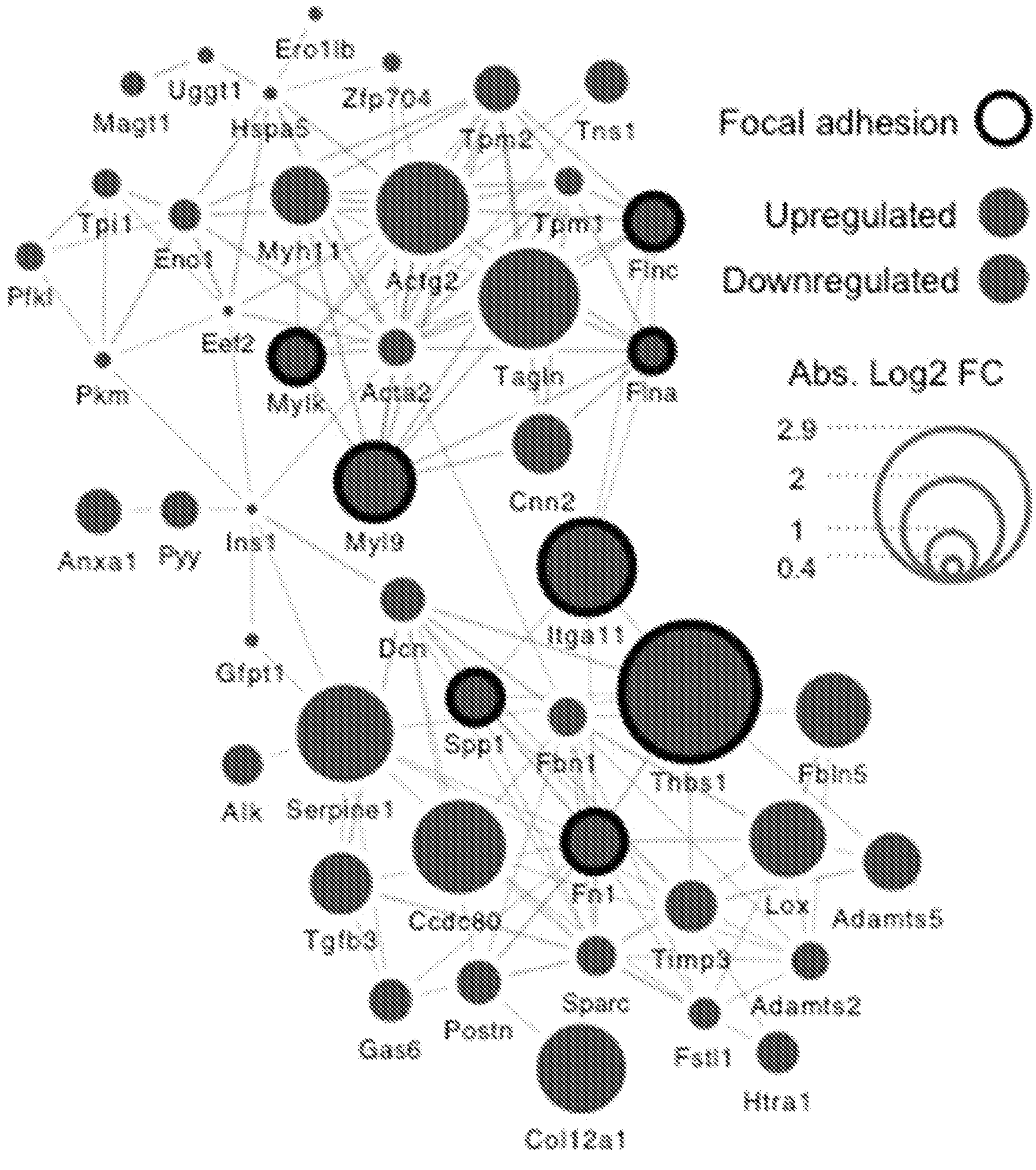

Ero1lb
Uggt1
Zfp704
Magt1
Hspa5
Tpm2
Tns1
Focal adhesion
Tpi1
Tpm1
Upregulated
Pfkl
Eno1
Myh11
Actg2
Flnc
Downregulated
Eef2
Pkm
Mylk
Acta2
Tagln
Flna
Abs. Log2 FC
2.9
2
1
0.4
Cnn2
Anxa1
Pyy
Ins1
Myl9
Itga11
Dcn
Gfpt1
Spp1
Fbn1
Thbs1
Fbln5
Alk
Serpine1
Fn1
Lox
Adamts5
Tgfb3
Ccdc80
Timp3
Sparc
Adamts2
Gas6
Postn
Fstl1
Htra1
Col12a1

FIG. 3D

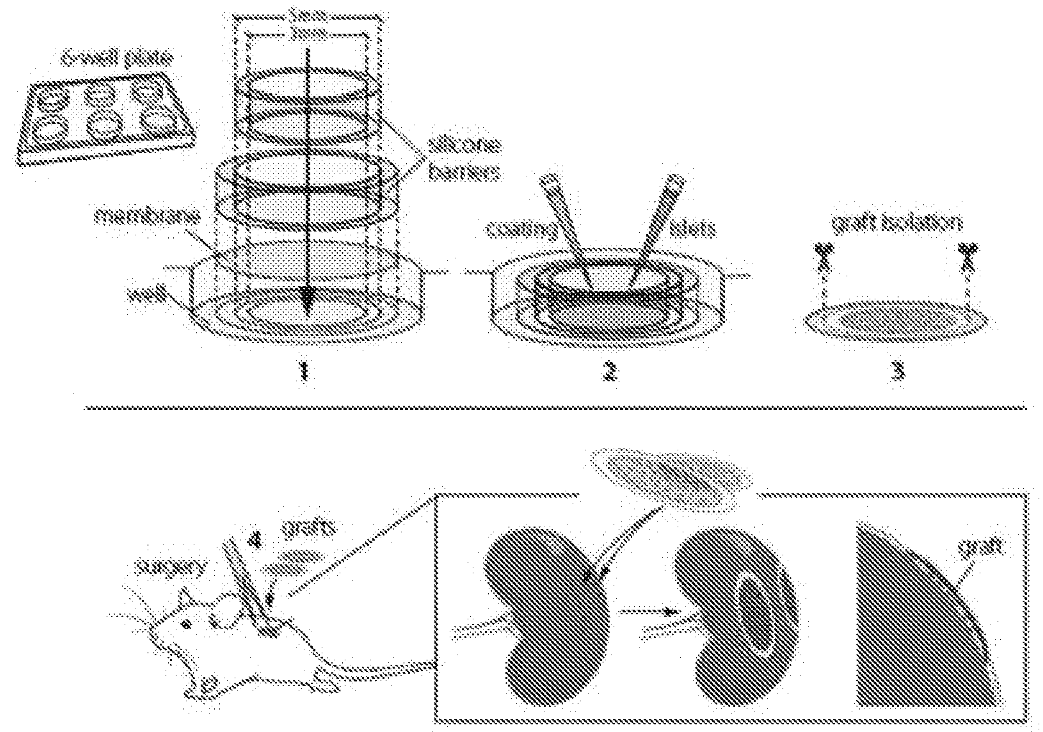

6-well plate
silicone barriers
membrane
well
coating
islets
graft isolation
1
2
3
surgery
4
grafts
graft

FIG. 4A

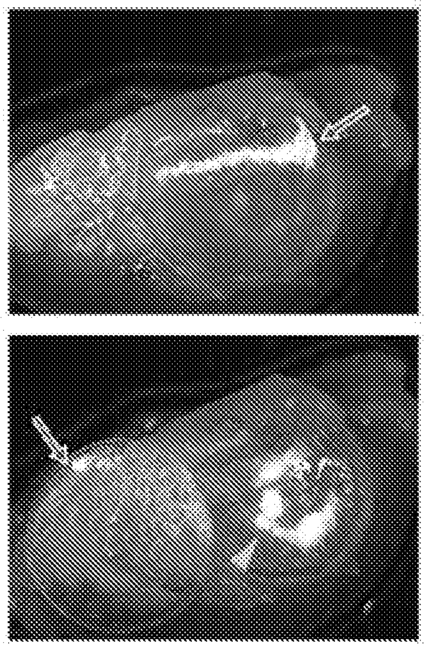


FIG. 4B

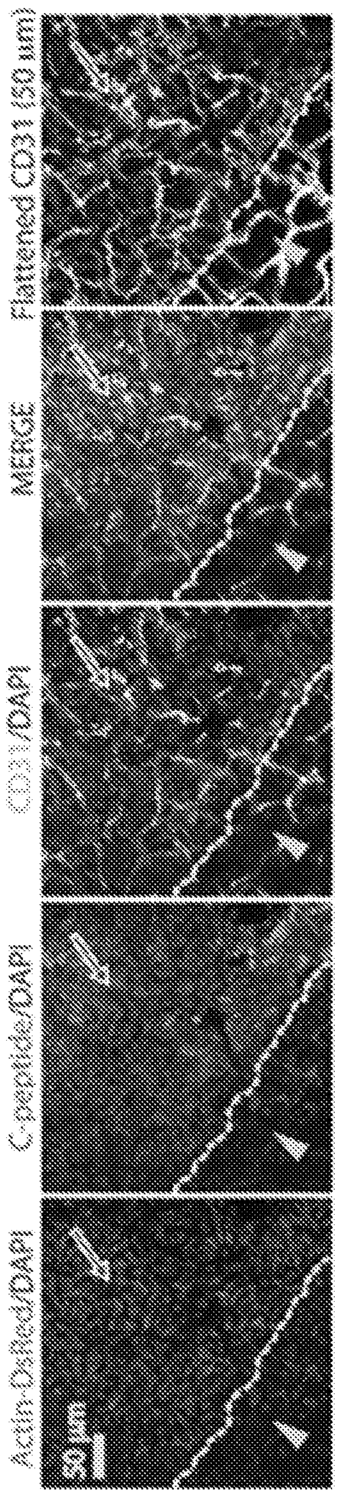

Actin-DsRed/DAPI
C-peptide/DAPI
CD31/DAPI
MERGE
Flattened CD31 (50 um)
50 µm

FIG. 4C

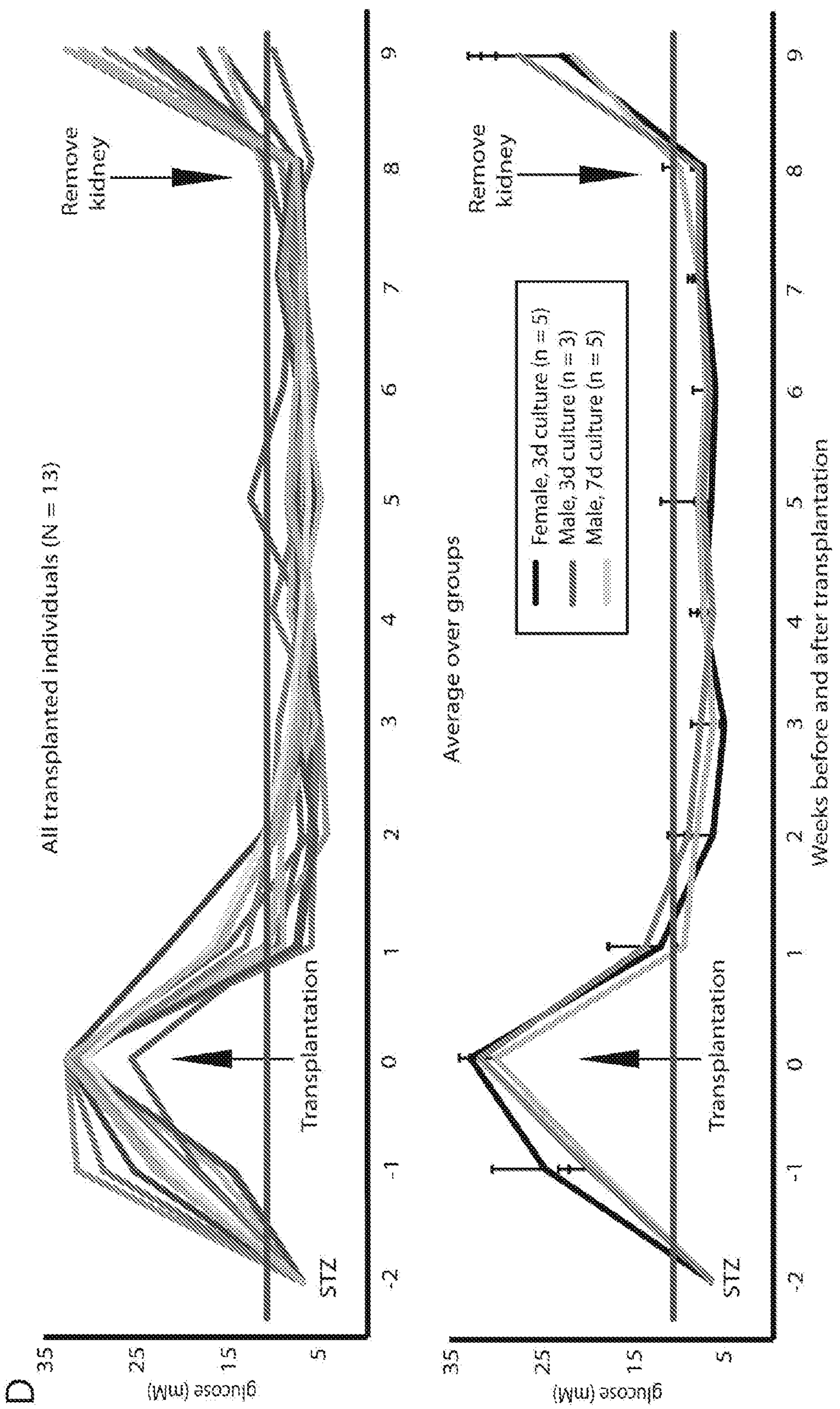

D
All transplanted individuals (N = 13)
glucose (mM)
35
25
15
5
STZ
Transplantation
Remove kidney
-2
-1
0
1
2
3
4
5
6
7
8
9
Average over groups
Female, 3d culture (n = 5)
Male, 3d culture (n = 3)
Male, 7d culture (n = 5)
Weeks before and after transplantation

FIG. 4D

FIG. 4E

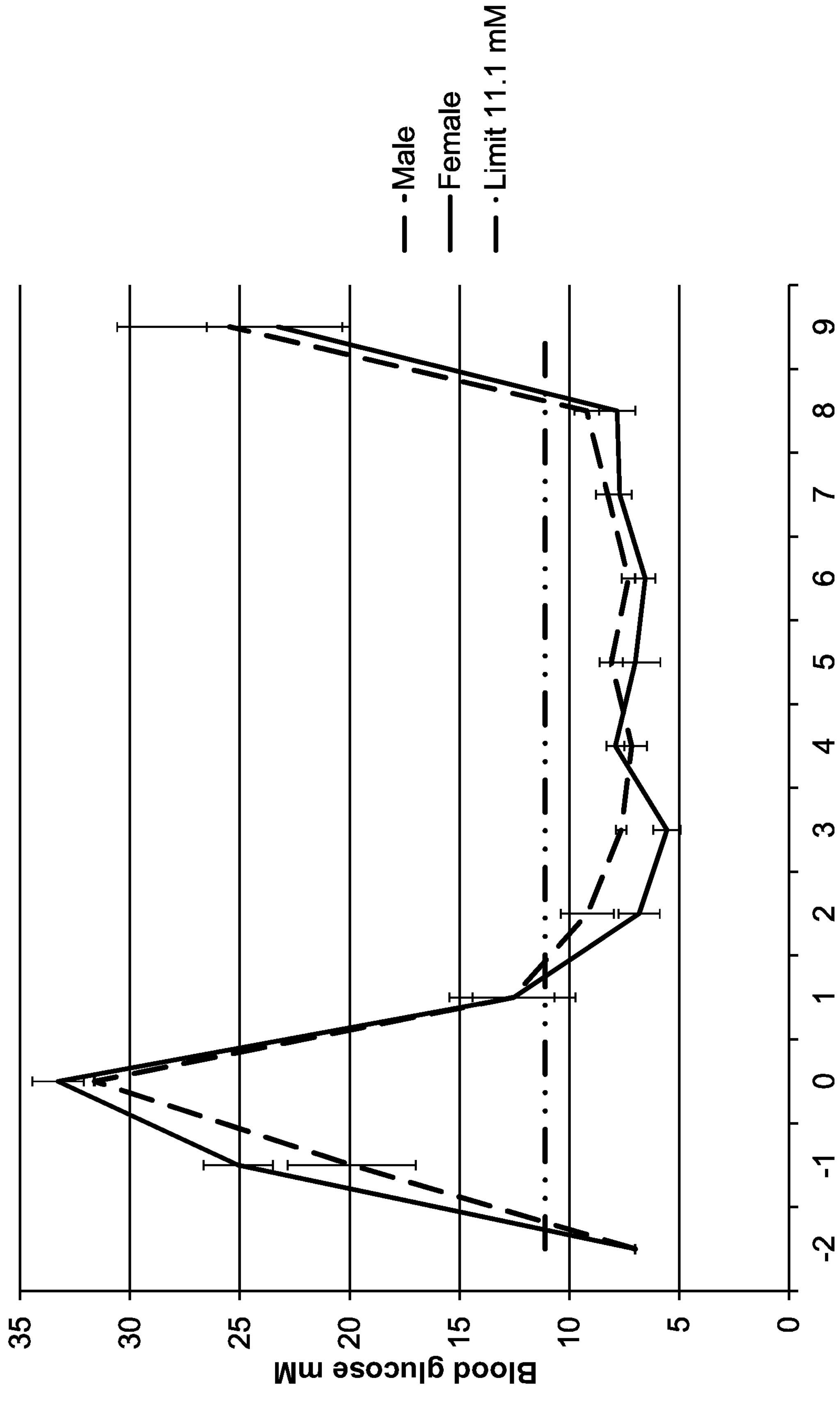
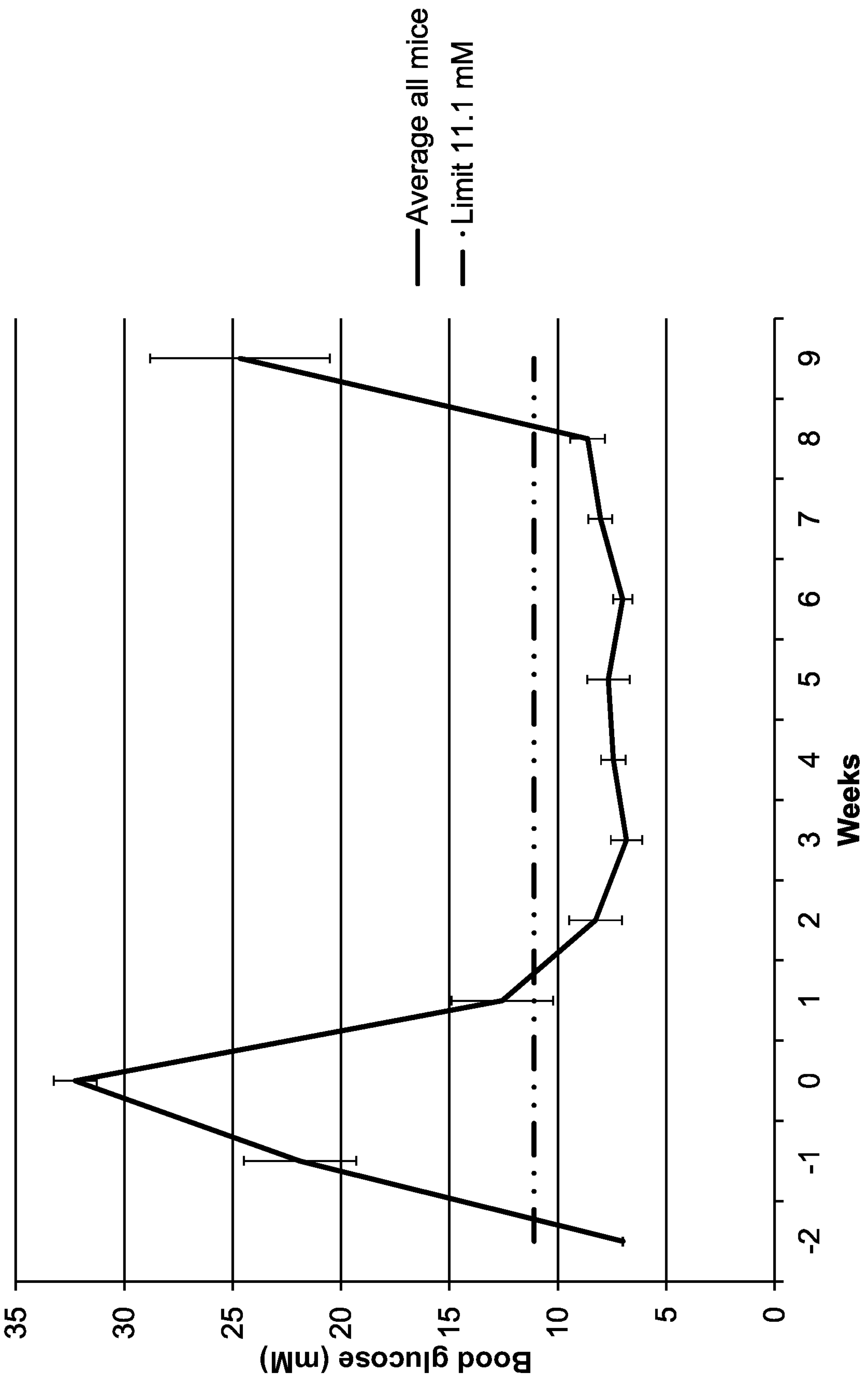

Male
Female
Limit 11.1 mM
Blood glucose mM
35
30
25
20
15
10
5
0
Weeks
-2
-1
0
1
2
3
4
5
6
7
8
9

FIG. 4F


Average all mice
Limit 11.1 mM
Bood glucose (mM)
35
30
25
20
15
10
5
0
Weeks
-2
-1
0
1
2
3
4
5
6
7
8
9

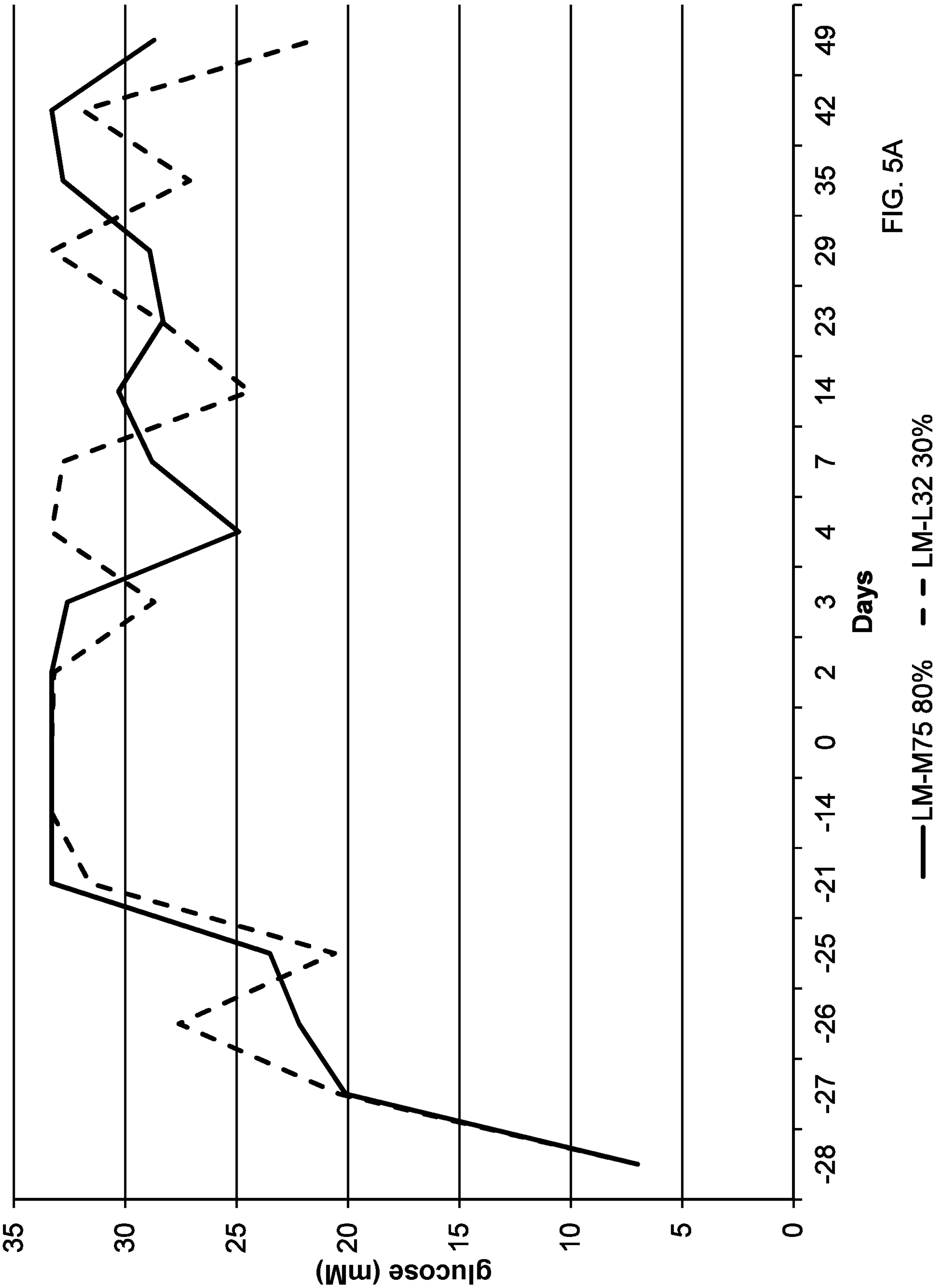

glucose (mM)
35
30
25
20
15
10
5
0
-28
-27
-26
-25
-21
-14
0
2
3
4
7
14
23
29
35
42
49
Days
LM-M75 80%
LM-L32 30%

FIG. 5A

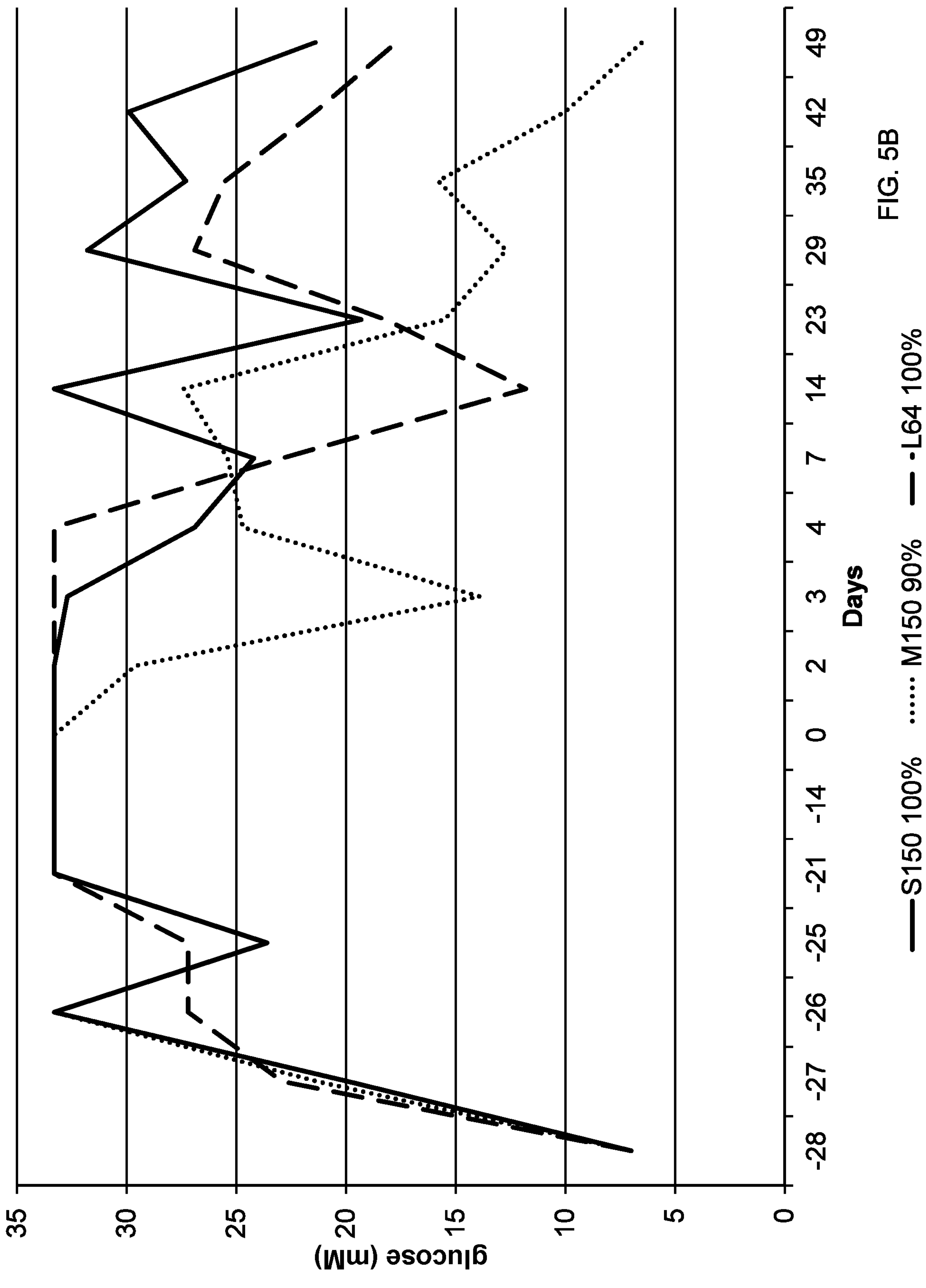

glucose (mM)
35
30
25
20
15
10
5
0
-28
-27
-26
-25
-21
-14
0
2
3
4
7
14
23
29
35
42
49
Days
S150 100%
M150 90%
L64 100%

FIG. 5B

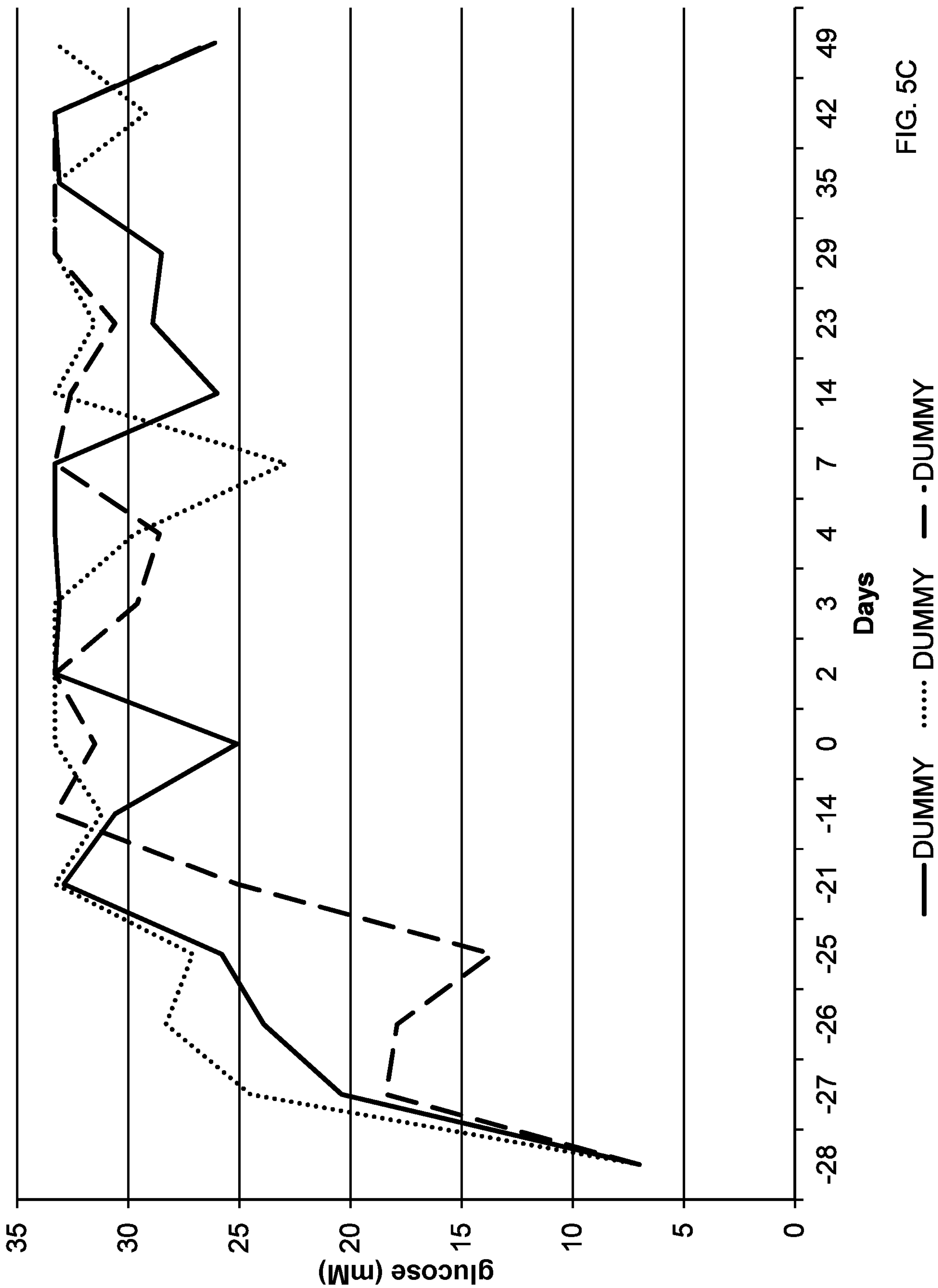

glucose (mM)
35
30
25
20
15
10
5
0
Days
-28
-27
-26
-25
-21
-14
0
2
3
4
7
14
23
29
35
42
49
DUMMY
DUMMY
DUMMY

FIG. 5C

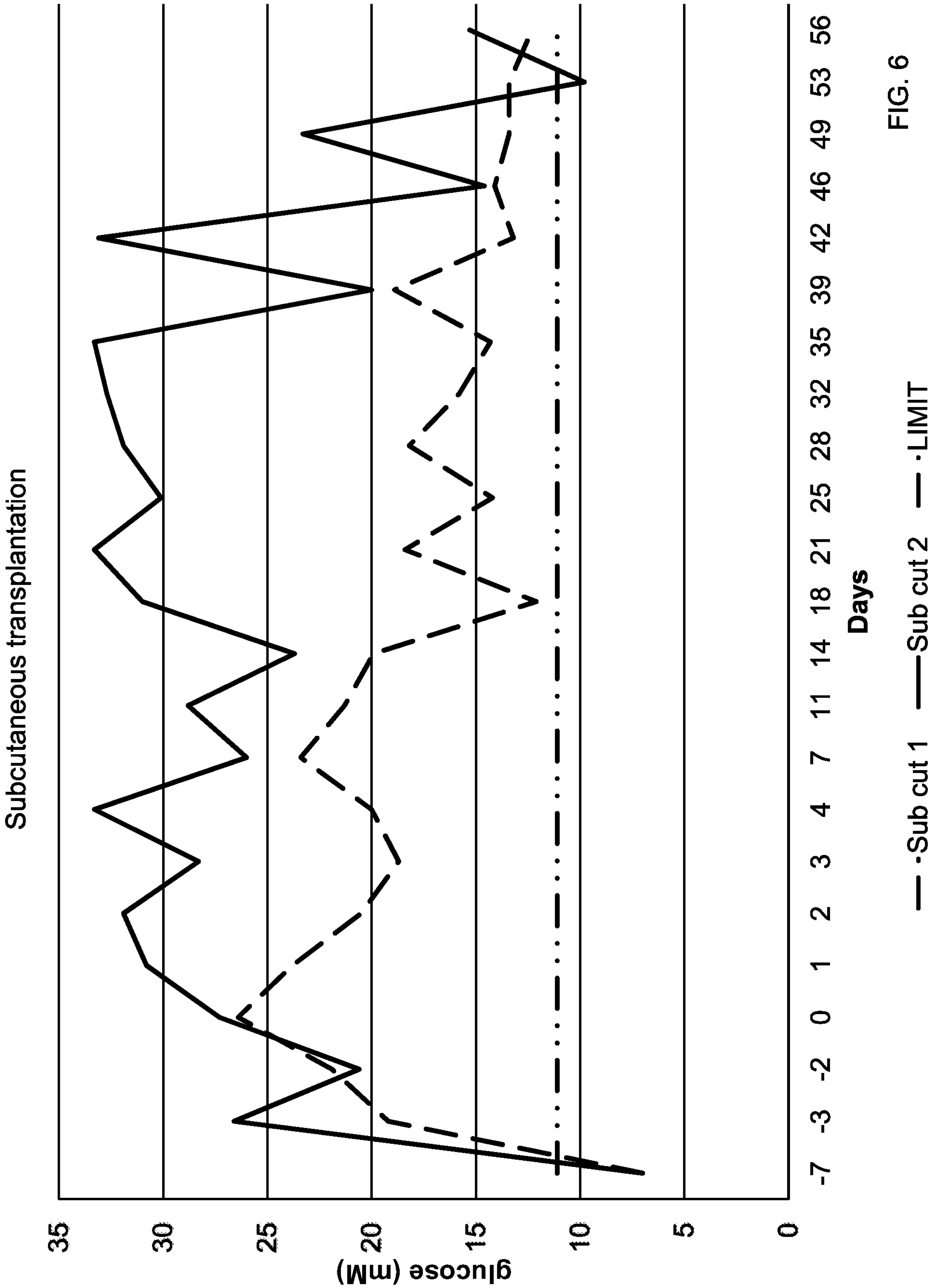

Subcutaneous transplantation
glucose (mM)
35
30
25
20
15
10
5
0
Days
-7 -3 -2 0 1 2 3 4 7 11 14 18 21 25 28 32 35 39 42 46 49 53 56
Sub cut 1
Sub cut 2
LIMIT

FIG. 6

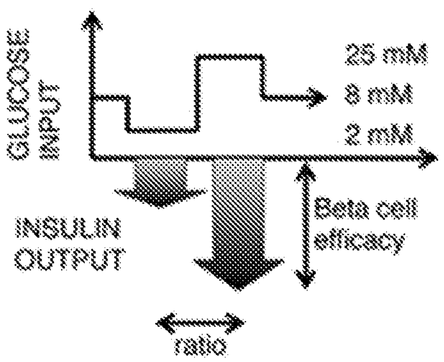

GLUCOSE INPUT
25 mM
8 mM
2 mM
INSULIN OUTPUT
Beta cell efficacy
ratio

FIG. 7A

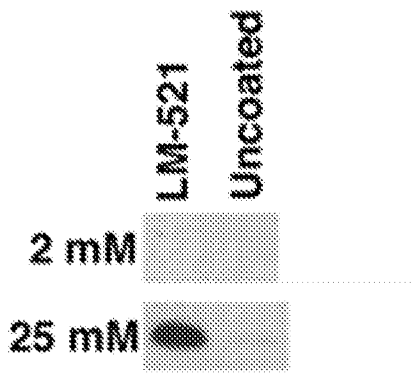

LM-521
Uncoated
2 mM
25 mM

FIG. 7B

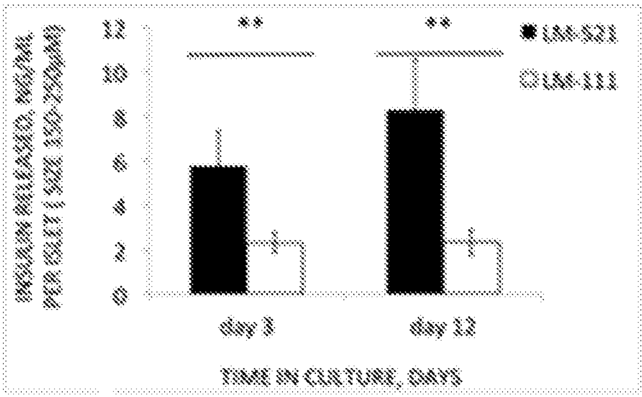


FIG. 7C

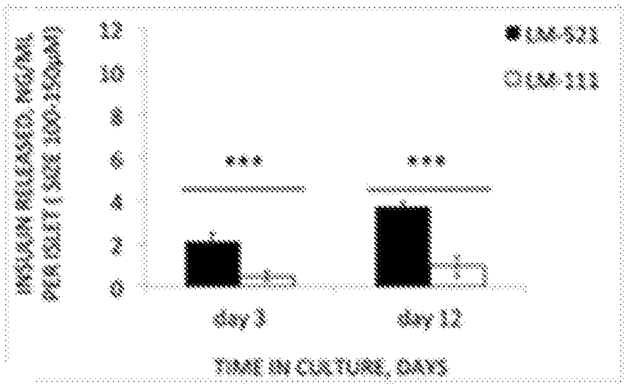


FIG. 7D

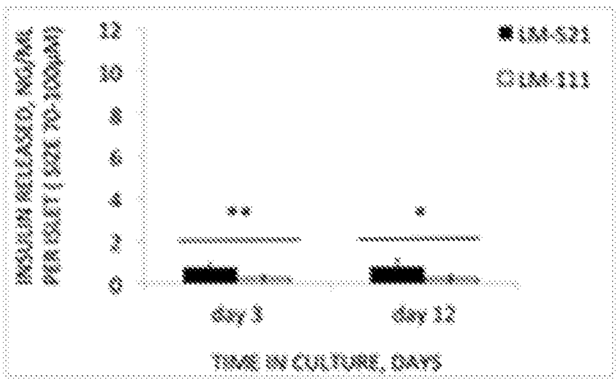


FIG. 7E

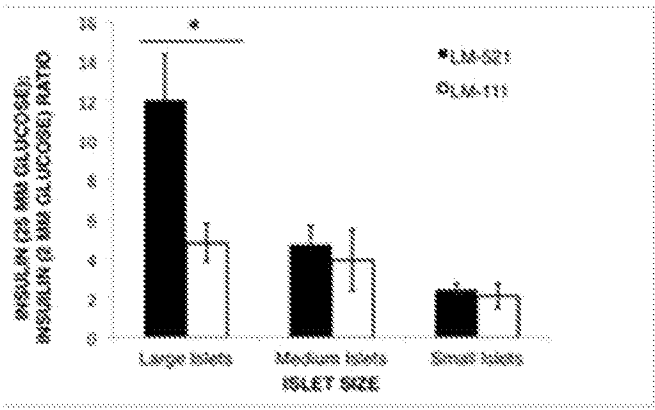

*
LM-521
LM-111
Large islets
Medium islets
Small islets
ISLET SIZE

FIG. 7F

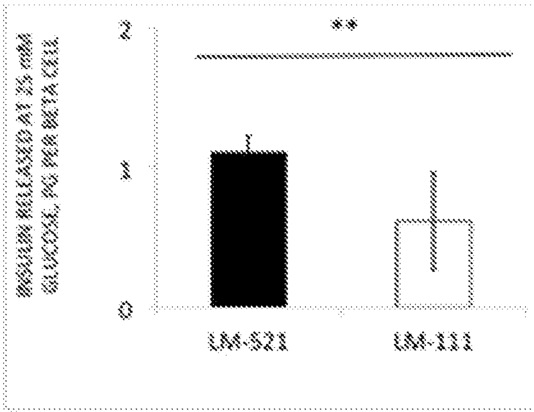

INSULIN RELEASED AT 25 mM GLUCOSE, PG PER BETA CELL
2
1
0
**
LM-521
LM-111

FIG. 7G

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SG2018/050567

A. CLASSIFICATION OF SUBJECT MATTER

C12N 5/071 (2010.01) *A61K 35/39 (2015.01)* *A61P 3/10 (2006.01)*

According to International Patent Classification (IPC)

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

FAMPAT/MEDLINE/EMBASE/BIOSIS/CAPLUS: expand, pancreatic islets, laminin, matrix, device, transplant, diabetes and related terms.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97/12961 A2 (DESMOS, INC.) 10 April 1997 whole document, particularly abstract and Examples 1-10	1-30
X	WO 97/37003 A1 (DESMOS, INC.) 9 October 1997 whole document, particularly abstract and Examples 1-6	1-30
X	WO 2013/156855 A1 (BIOLAMINA AB) 24 October 2013 para [0095]- [0107]	1-30

☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

*Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
31/01/2019 *(day/month/year)*	*12/02/2019* *(day/month/year)*

Name and mailing address of the ISA/SG	Authorized officer
IPOS INTELLECTUAL PROPERTY OFFICE OF SINGAPORE **Intellectual Property Office of Singapore** 51 Bras Basah Road #01-01 Manulife Centre Singapore 189554 Email: pct@ipos.gov.sg	Fu Yilong (Dr) IPOS Customer Service Tel. No.: (+65) 6339 8616

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SG2018/050567

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	YAMASHITA S. ET AL., Human Laminin Isotype Coating for Creating Islet Cell Sheets. *Cell Med*, 21 August 2015, Vol. 8, No. 1-2, pages 39-46 [Retrieved on 2019-01-31] <DOI: 10.3727/215517915X689029> whole document	1-30
P,X	SIGMUNDSSON K. ET AL., Culturing functional pancreatic islets on α5-laminins and curative transplantation to diabetic mice. *Matrix Biol.*, 27 March 2018, Vol. 70, pages 5-19 [Retrieved on 2019-01-31] <DOI: 10.1016/J.MATBIO.2018.03.018> whole document	1-30

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/SG2018/050567

Note: This Annex lists known patent family members relating to the patent documents cited in this International Search Report. This Authority is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 97/12961 A2	10/04/1997	DE 69630972 T2	07/04/2005
		AU 7367296 A	28/04/1997
		CA 2233859 A1	10/04/1997
		US 5681587 A	28/10/1997
		EP 0859830 A2	26/08/1998
		ES 2211988 T3	16/07/2004
		JP H11513253 A	16/11/1999
		AT 255631 T	15/12/2003
WO 97/37003 A1	09/10/1997	CA 2250938 A1	09/10/1997
		US 5672361 A	30/09/1997
		AU 2428097 A	22/10/1997
		JP 2000508890 A	18/07/2000
		EP 0914414 A1	12/05/1999
WO 2013/156855 A1	24/10/2013	EP 3282009 A1	14/02/2018
		DK 2838992 T3	05/02/2018
		EP 2838992 A1	25/02/2015
		US 2013/0280750 A1	24/10/2013
		US 2017/0067016 A1	09/03/2017