GLP-1 Receptor Expression Within the Human Heart

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Glucagonlike peptide-1 receptor (GLP-1R) agonists, which are used to treat type 2 diabetes and obesity, reduce the rates of myocardial infarction and cardiovascular death. GLP-1R has been localized to the human sinoatrial node; however, its expression in ventricular tissue remains uncertain. Here we studied GLP-1R expression in the human heart using GLP-1R–directed antisera, quantitative polymerase chain reaction (PCR), reverse transcription PCR to detect full-length messenger RNA (mRNA) transcripts, and *in situ* hybridization (ISH). *GLP1R* mRNA transcripts, encompassing the entire open reading frame, were detected in all four cardiac chambers from 15 hearts at levels approximating those detected in human pancreas. In contrast, cardiac *GLP2R* expression was relatively lower, and cardiac *GCGR* expression was sporadic and not detected in the left ventricle. *GLP1R* mRNA transcripts were not detected in RNA from human cardiac fibroblasts, coronary artery endothelial, or vascular smooth muscle cells. Human Brunner glands and pancreatic islets exhibited GLP-1R immunopositivity and abundant expression of *GLP1R* mRNA transcripts by ISH. *GLP1R* transcripts were also detected by ISH in human cardiac sinoatrial node tissue. However, definitive cellular localization of *GLP1R* mRNA transcripts or immunoreactive GLP-1R protein within human cardiomyocytes or cardiac blood vessels remained elusive. Moreover, validated GLP-1R antisera lacked sufficient sensitivity to detect expression of the endogenous islet or cardiac GLP-1R by Western blotting. Hence, although human cardiac ventricles express the *GLP1R*, the identity of one or more ventricular cell type(s) that express a translated GLP1R protein requires further clarification with highly sensitive methods of detection. (***Endocrinology*** **159: 1570–1584, 2018)**

Incretin hormones, principally glucose-dependent insulinotropic polypeptide and glucagonlike peptide-1 (GLP-1), are secreted from the gut after meal ingestion and amplify glucose-dependent insulin secretion (1). GLP-1 also inhibits glucagon secretion and gastric emptying and promotes satiety; these actions underly the therapeutic use of GLP-1 receptor (GLP-1R) agonists in the treatment of type 2 diabetes (T2D) (2, 3). A single GLP-1R agonist, liraglutide, has also been approved for the treatment of obesity (4); an oral form of semaglutide has entered phase 3 clinical testing (5); and multiple unimolecular agonists that enhance GLP-1 action are under clinical development (6). Hence, there is ongoing interest in the translational mechanisms of GLP-1 action.

The actions of GLP-1 are mediated through a single GLP-1R, a member of the class B family of G protein–coupled receptors (7). The GLP-1R is widely expressed outside the pancreas, including neurons within the enteric, central, and peripheral nervous system; kidney; lung; the immune system; and diverse cell types within the stomach and the small and large bowel (8–10). GLP-1R has also been identified in the cardiovascular system; within blood vessels, vascular smooth muscle cells, and endothelial cells; and in the heart (11, 12).

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Abbreviations: BHK, baby hamster kidney; CFPAC-1, cystic fibrosis pancreatic adenocarcinoma; GLP-1, glucagonlike peptide-1; GLP-1R, glucagonlike peptide-1 receptor; ISH, *in situ* hybridization; PCR, polymerase chain reaction; qPCR, quantitative polymerase chain reaction; RT-PCR, reverse transcription polymerase chain reaction; T2D, type 2 diabetes.

Consistent with the detection of *GLP1R* messenger RNA (mRNA) transcripts and protein in human heart and blood vessels, GLP-1R agonists exert multiple actions in the cardiovascular system, including induction of heart rate, vasodilation, and reduction of blood pressure in the setting of hypertension (13). Activation of GLP-1R signaling rapidly improves ventricular function in the context of transient ischemia in preclinical and clinical studies (14) and reduces infarct size in experimental models of ischemia secondary to coronary artery ligation (15, 16). Short-term administration of GLP-1R agonists for a few hours also reduces the extent of reperfusion injury in the myocardium of patients with ST-segment elevation myocardial infarction (17). Nevertheless, the mechanisms and cell types through which GLP-1R signaling reduces injury of the cardiac ventricles remains incompletely understood.

The requirement of regulatory agencies mandating assessment of the cardiovascular safety of newer drugs approved for the treatment of T2D has further enhanced interest in understanding how GLP-1R agonists might regulate cardiovascular function. The first reported outcome study for drugs in this class examined the cardiovascular safety of lixisenatide in human subjects with T2D and recent acute coronary syndrome; lixisenatide did not significantly alter the rate of major adverse cardiovascular events (18). The cardiovascular safety of once-weekly exenatide was also recently established in over 14,000 subjects with T2D, of whom more than 70% had established cardiovascular disease (19). In contrast, two studies examining the cardiovascular safety of liraglutide and semaglutide demonstrated a reduction in the rate of death from cardiovascular causes, nonfatal myocardial infarction, or nonfatal stroke in subjects with T2D (20, 21).

The demonstration that some GLP-1R agonists produce cardioprotective actions in human subjects raises important questions surrounding the cardiovascular biology of the human GLP-1R. Although the expression and function of the GLP-1R in the cardiovascular system has been extensively examined in preclinical studies, the precise localization of *GLP1R* expression in the human heart remains uncertain. Wallner *et al.* (22) detected *GLP1R* mRNA transcripts using reverse transcription polymerase chain reaction (RT-PCR) in RNA from human atrium and ventricle. Exenatide increased contractility in isolated trabeculae from the atrium but not from the majority of ventricular preparations examined (22). Consistent with the actions of GLP-1R agonists to increase heart rate (15), a well-characterized monoclonal antibody detected GLP-1R–immunopositive cells within the sinoatrial node of nonhuman primates and a single human heart (23).

Despite the growing translational interest in understanding sites and mechanisms of GLP-1 action in the human heart, few studies have comprehensively assessed GLP-1R expression in human cardiac tissue. Moreover, detection of the immunoreactive GLP-1R has proven challenging due to the suboptimal sensitivity and specificity of the available reagents (24, 25). To determine the localization of *GLP1R* expression in the human heart, we studied *GLP1R* expression in heart samples available from two different human tissue biobanks. We also assessed the sensitivity and specificity of two GLP-1R antisera known to recognize the human GLP-1R. Here we report that *GLP1R* mRNA transcripts encoding the entire GLP-1R open reading frame were consistently detected in biopsy samples obtained from all four chambers from 15 different human hearts. Despite analysis of histological sections from an independent group of 35 human hearts using a combination of *in situ* hybridization (ISH) and immunohistochemistry, we were unable to identify the precise cellular localization of human ventricular GLP-1R expression.

Materials and Methods

Human tissue samples

Human heart samples (left atrium, right atrium, left ventricle, right ventricle) for RNA and protein isolation were acquired from the University of Pennsylvania Heart BioBank. Tissues from failing human hearts were obtained from patients receiving heart transplantation at the University of Pennsylvania. The use of human heart tissue for research was approved by the University of Pennsylvania's Institutional Review Board. Nonfailing heart tissue was obtained from deceased organ donors through the Gift-of-Life donor program in Philadelphia. Prospective informed consent for research use of heart tissue was obtained from all transplant recipients and from the appropriate next of kin for the organ donors. All human tissues received *in situ* high-potassium cardioplegia at the time of tissue procurement. Conventions for cardiac tissue sampling were as follows: left ventricle—free wall from a noninfarcted region, no septum, and predominantly anterior wall midway between the apex and base; right ventricle—anterior free wall in a region containing minimal epicardial fat; left atrium—free wall, no septum, and no left atrial appendage; right atrium—free wall, no septum, and no right atrial appendage. A description of these human heart samples is provided in Supplemental Table 1 and in Ussher *et al.* (26). Human cardiac tissues were pulverized in liquid nitrogen and stored at −80°C prior to RNA or protein extraction. Human adipose tissues for RNA isolation were obtained from abdominal wall samples that were removed from patients undergoing breast reconstruction surgery via tram flap and were provided by Dr. John Semple [University Health Network (UHN), Toronto, ON, Canada]. Human intestinal tissue (colon) was obtained from the Mount Sinai Hospital tissue bank after research ethics approval. Human cardiac tissue sections for immunohistochemistry and ISH experiments were obtained from a total of 35 different subjects from the UHN Rapid Autopsy Program as approved by the UHN Research Ethics Board. Supplemental Table 2 provides a list of these human cardiac tissues. Human Brunner glands, pancreas tissue, and cardiac atrial sections were provided by Dr. Catherine Streutker (St. Michael's Hospital, Department of Surgical Pathology,

Toronto, ON, Canada). Human islet samples (>95% purity) were generously provided by Dr. Patrick MacDonald (Alberta Diabetes Institute, University of Alberta, Edmonton, AB, Canada). Human islet donor information is listed in Supplemental Table 3.

Human RNA samples

Human RNA samples were purchased from the following sources: adipose (catalog no. R1234003-10, BioChain, Newark, CA), bone marrow (catalog no. R1234024-10, BioChain; catalog no. HR-704, Zyagen, SanDiego, CA; catalog no. 636591, Takara Bio, Mountain View, CA), coronary artery endothelial cells (catalog no. 300-R10a, Cell Applications Inc., San Diego, CA), human coronary artery smooth muscle cells (catalog no. 350-R10a, Cell Applications Inc.), peripheral blood leukocyte (catalog no. 636592, Takara Bio; catalog no. R1234148-10, BioChain), pericardium (catalog no. R1234133-50, BioChain), liver (catalog no. AM7960, Thermo Fisher, Markham, ON, Canada; catalog no. 540017, Agilent Tech., Santa Clara, CA; catalog no. 636531, Clontech, Mountain View, CA). Human pancreas RNA was kindly provided by Dr. Maria Chrisina Nostro (Department of Physiology, University Health Network, University of Toronto, Toronto, ON, Canada).

Cell lines

Baby hamster kidney (BHK) fibroblast cells stably expressing the human GLP-1 receptor at high, low, and ultra-low levels of expression (BHK clones #12A, #13, and #27, respectively) (27, 28) were provided by Novo Nordisk (Bagsvaerd, Denmark). BHK cells transfected with pcDNA3.1 (Thermo Fisher) were generated as described (29). The human cystic fibrosis pancreatic adenocarcinoma (CFPAC-1) cell line was purchased from American Type Culture Collection (CRL-1918; ATCC, Manassas, VA). Human cardiac fibroblasts were purchased from ScienCell Research Laboratories (San Diego, CA).

RNA isolation and analysis

Total RNA was extracted from tissues or cells using Tri Reagent (Molecular Research Center Inc., Cincinnati, OH). cDNA was synthesized from DNase I–treated (Thermo Fisher) total RNA using random hexamers and Superscript III (Thermo Fisher). Real-time quantitative polymerase chain reaction (qPCR) was carried out using a QuantStudio 5 System and TaqMan Gene Expression Assays (Thermo Fisher). Relative mRNA levels were quantified using the $2^{-\Delta Ct}$ method. A list of qPCR primers and assay identification numbers is provided in Supplemental Table 4. Polymerase chain reaction (PCR) products encompassing the majority of the human *GLP1R*, *GCGR*, *GLP2R*, or *DPP4* coding regions were amplified from cDNA, separated on an agarose gel, transferred to a nylon membrane, and hybridized with internal human-specific ^{32}P-labeled oligonucleotide probes for detection of *GLP1R*, *GCGR*, *GLP2R*, or *DPP4* mRNA transcripts. Blots were exposed to a Storm 860 Phosphor Screen (Biorad, Mississauga, ON, Canada) and visualized using Quantity One imaging software (Bio-Rad). PCR oligonucleotide primer sequences are listed in Supplemental Table 4.

Western blot analyses

Whole tissue (human heart chambers and pancreatic islets) and cell line (BHK fibroblast and CFPAC-1) protein extracts were prepared by homogenization in PBS supplemented with 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate, and protease inhibitor cocktail (Sigma-Aldrich, Oakville, ON, Canada). Extracts were cleared by centrifugation and then treated for 1 h at 37°C with sample buffer containing β-mercaptoethanol. Protein (30 to 40 μg) was resolved by discontinuous sodium dodecyl sulfate polyacrylamide gel electrophoresis and then electrotransferred onto Amersham Protran nitrocellulose membrane (GE Health Care Life Sciences, Mississauga, ON, Canada) using standard procedures. Immunoprecipitations were performed using 1 mg/mL of cell or tissue protein extract and 2 μg/mL of rabbit polyclonal anti-human GLP-1R antibody [Novus 19400002; Research Resource Identifier (RRID) AB_10004637; Bio-Techne, Oakville, ON, Canada] overnight at 4°C. Immune complexes were captured using Protein A Plus Agarose (Thermo Fisher Scientific) and then treated with sample buffer as described previously prior to sodium dodecyl sulfate polyacrylamide gel electrophoresis and electrotransfer to Protran membrane. Blots were probed at room temperature with antibodies at 1:1000 dilutions in 5% skim milk in Tris-buffered saline containing 0.2% Tween 20. Antigen-antibody complexes were visualized with a secondary antibody conjugated to horseradish peroxidase and an enhanced chemiluminescence kit (SuperSignal WestPico; Thermo Fisher Scientific). Chemiluminescence detection was performed on a Kodak Image Station 4000MM Pro (Carestream Health Canada, Vaughan ON, Canada). The following antibodies to the GLP-1R were used to probe blots: mouse monoclonal 3F52 (DSHB; RRID AB_2618100; University of Iowa, Iowa City, IA), which recognizes the human GLP-1R, and rabbit polyclonal Novus 19400002. The rabbit polyclonal antibodies anti-Akt and anti-Erk1/2 were from Cell Signaling Technology (Whitby, ON, Canada). Antibodies used in this study are listed in Table 1.

GLP-1R immunohistochemistry

Immunohistochemistry was performed on formalin-fixed tissues as described (23). Tissue sections were incubated with the primary GLP-1R antibody (Mab 3F52, 0.83 or 5 μg/mL final concentration) for 2 hours at room temperature and counterstained with Mayer Hematoxylin (Agilent Tech.). Slides were imaged using Leica IM50 Image Manager Software version 1.20 (Leica Microsystems Inc., Concord, ON, Canada). Sections containing human Brunner glands or human pancreas were simultaneously analyzed as positive controls.

Table 1. Antibodies Used

Peptide/Protein Target	Antigen Sequence	Name of Antibody	Supplier	Monoclonal or Polyclonal	Dilution Used	RRID
GLP-1R	N/A	Novus 19400002	Novus Biologicals	Polyclonal	1:1000	AB_10004637
GLP-1R	N/A	Novo 3F52	Novo Nordisk	Monoclonal	1:1000	AB_2618100

Abbreviations: N/A, not applicable.

ISH

Tissues were cut into 5-μm sections and mounted onto Superfrost Plus (Thermo-Fisher Scientific) glass slides. ISH was performed using the RNAscope 2.5 HD Detection Reagent kit (Advanced Cell Diagnostics Inc., Newark, CA) and human GLP-1R (Hs-GLP1R; catalog no. 519821) or PECAM1 (Hs-PECAM1-No-XMm-01; catalog no. 455931) specific hybridization probes (Advanced Cell Diagnostics Inc.) according to the manufacturer's instructions and counterstained with Mayer Hematoxylin (Agilent Tech.). Slides were imaged using Leica IM50 Image Manager Software version 1.20 (Leica Microsystems Inc., Concord, ON, Canada). Sections containing human Brunner glands or human pancreas were simultaneously analyzed by ISH as positive controls.

Statistical analyses

Data are presented as mean ± standard error. Statistical significance was determined by two-tailed Student *t* test using Prism version 5.02 software (GraphPad, San Diego, CA). A *P* value ≤ 0.05 was considered statistically significant.

Results

To ascertain the relative levels of *GLP1R* mRNA transcripts in human tissues, we first quantified *GLP1R* mRNA transcripts using qPCR (Fig. 1). *GLP1R* mRNA transcripts were detected in all four chambers of the human heart at levels comparable to those detected in RNA from human pancreas but at lower levels than those detected in RNA from human islets (Fig. 1A). Much lower (100- to 1000-fold less abundant) levels of *GLP1R* mRNA transcripts were detected in RNA from human bone marrow, peripheral blood leukocytes, and pericardium (Fig. 1A). In contrast, we did not detect *GLP1R* mRNA transcripts in RNA from human coronary artery endothelial cells, coronary artery vascular smooth muscle cells, or human cardiac fibroblasts (Fig. 1A and 1B and Supplemental Fig. 1).

To assess the qualitative integrity and to verify the origin of the RNA isolated from the human cells studied in Fig. 1A, we analyzed the expression of *GLP1R* and key genes associated with different cell lineages. We detected mRNA transcripts for *THY1* in cells from bone marrow; *VIM* mRNA transcripts in RNA from pericardium and cardiac fibroblasts; *TEK* and *ICAM1* expression in RNA from endothelial cells; *SMTN*, *ENG*, and *TAGLN* in vascular smooth muscle RNA; and *PTPRC* transcripts in RNA from peripheral blood leukocytes (Fig. 1B). Collectively, these analyses are consistent with the designated cellular sources of the human RNA samples.

qPCR is sufficiently sensitive to detect very low levels of a partial cDNA fragment but does not provide information about the expression of a full-length mRNA transcript. Accordingly, we assessed *GLP1R* expression by conventional PCR using primers spanning the entire coding region of the GLP1R mRNA and protein. A full-length *GLP1R* mRNA transcript was uniformly detected in RNA from all four cardiac chambers in all 15 human hearts examined (Fig. 2). In contrast, mRNA transcripts for the structurally related glucagon receptor (*GCGR*), which is expressed and functional in the murine adult ventricles (30), were not detected in most of the hearts examined and were absent from the left ventricle (Fig. 3A). Moreover, *GLP2R* mRNA transcripts were expressed at lower levels compared with *GLP1R* ($P < 0.05$ for relative mRNA levels of *GLP1R* vs *GLP2R* expression in all four heart chambers; Fig. 4 and data not shown) and were detected in some but not all RNA isolates from all four chambers of the human heart (Fig. 3B).

To further quantify the relative expression of class B G-protein–coupled receptors in the human heart, we compared levels of *GLP1R*, *GCGR*, *GIPR*, and *GLP2R*, with mRNA transcripts for *INSR*, *DPP4*, *HCN4*, *NPPA*, *MYL7*, *ADCY5*, *ADCY6*, *MAPK3*, and *CREB1* in RNA from 15 different human hearts (Fig. 4). The relative expression of the class B G-protein–coupled receptors examined, including *GLP1R*, was at least 10- to 100-fold lower relative to the abundance of the majority of mRNA transcripts examined, most of which are known to be expressed in cardiomyocytes (Fig. 4). Furthermore, considerable interindividual variation in relative *GLP1R* expression was detected in all four chambers (Fig. 4). For comparison, the relative expression of the insulin receptor mRNA (*INSR*), which encodes a transmembrane receptor protein known to be expressed and functional in ventricular cardiac myocytes, was 100- to 1000-fold higher than the level of *GLP1R* expression in the left ventricle (Fig. 4).

We next assessed whether a combination of immunohistochemistry and ISH would identify specific GLP-1R+ cell types within the human heart. We first examined the suitability of two different GLP-1R antibodies previously shown to detect the human GLP-1R, monoclonal antibody 3F52 (23) and the polyclonal antibody Novus 19400002 (31). Both antibodies detected the GLP-1R protein in Western blot analysis using BHK fibroblasts expressing high levels of a transfected human GLP-1 receptor cDNA (Fig. 5A, left panels, lane 2). In contrast, an immunoreactive GLP-1R was not detected in transfected BHK cells expressing relatively lower levels of transfected GLP-1R mRNA (Fig. 5A, lanes 3 and 4) or in CFPAC-1 cells previously shown to express a functional endogenous human GLP-1R (32) (Fig. 5A, lane 5). Neither antibody was sufficiently sensitive to enable detection of the endogenous GLP-1R in whole tissue protein extracts from isolated human islets (Fig. 5A, right panels) or in protein extracts from all four chambers of the human heart (Fig. 5B). Nonetheless, immunoprecipitation using the Novus antibody followed by Western blot with the 3F52 antibody detected GLP-1R in human islets (Fig. 6A) but not in human heart (Fig. 6B).

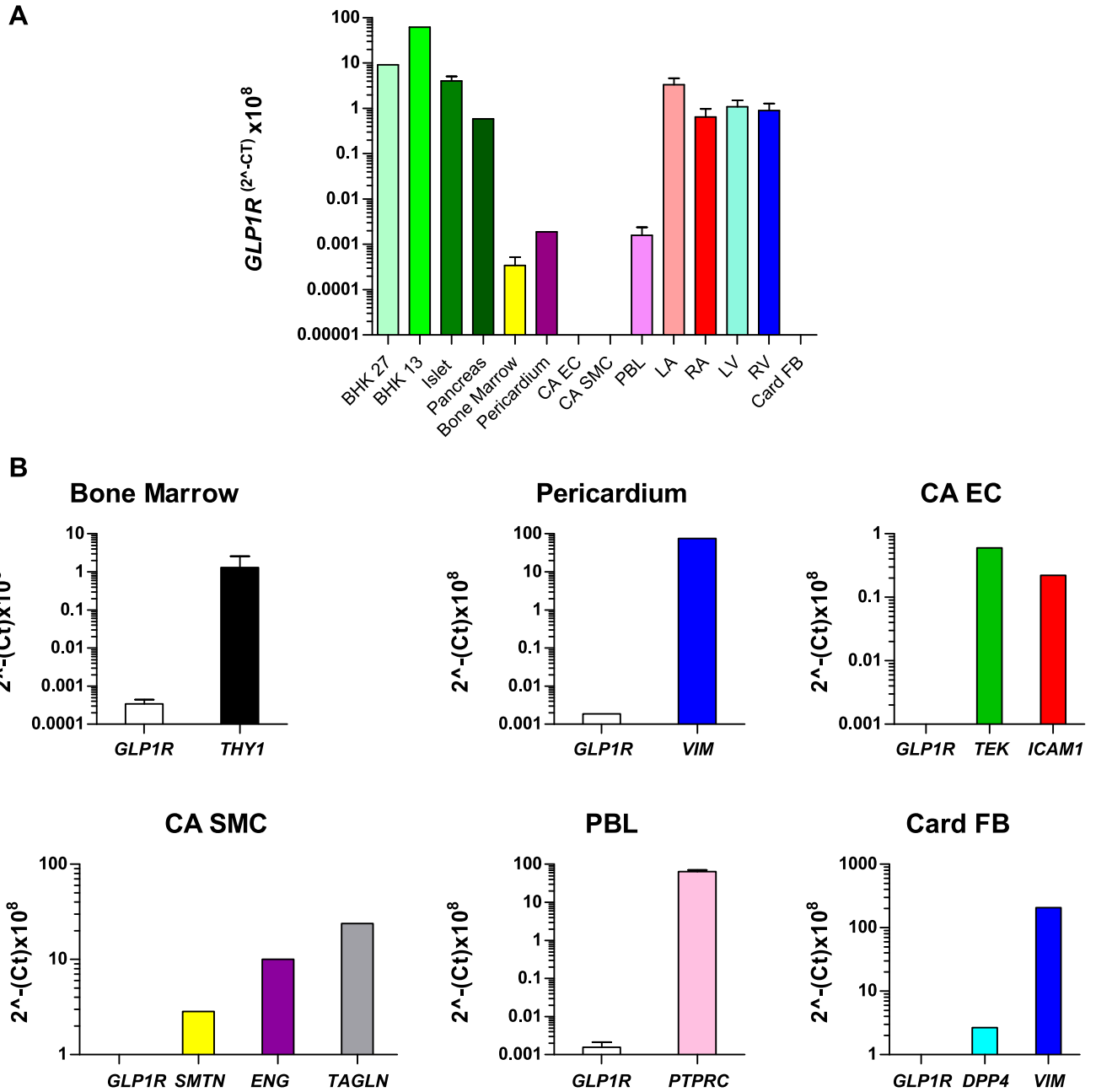

Figure 1. *GLP1R* mRNA transcript levels in the human heart are comparable to those in human pancreas and islets. (A) *GLP1R* mRNA levels were measured via qPCR analysis in multiple human tissues and in transfected BHK cells that express low levels of the human GLP-1R. For data represented without standard error bars, each single RNA sample was analyzed in duplicate; for isolates depicted with error bars, peripheral blood lymphocyte samples were analyzed in duplicate from two different sources, and at least three different samples were analyzed in duplicate by qPCR for RNAs from islet, bone marrow, left atria (LA), right atria (RA), left ventricle (LV), and right ventricle (RV). (B) qPCR analysis of *GLP1R* and tissue- or cell-type–specific gene expression in the indicated samples as confirmation of RNA/cDNA integrity. For (A) and (B), data are expressed as cycle threshold (Ct) values because none of the housekeeping genes examined (*ACTB*, *GPI*, *PSMB4*, *CHMP2A*, and *EMC7*) exhibited consistent expression levels in all tissues examined. Values are mean ± standard error (where appropriate); n = 1 to 3 samples per tissue. LA samples are from patients P01371, P01430, and P01504. RA samples are from patients P01262, P01371, and P01377. LV samples are from patients P01262, P01430, and P01371. RV samples are from patients P01262, P01371, and P01504 (see Supplemental Table 1 and Figure 4). Islet samples are from donors R177, R199, and R200 (see Supplemental Table 3). CA EC, coronary artery endothelial cells; CA SMC, coronary artery smooth muscle cells; PBL, peripheral blood lymphocytes; Card FB, cardiac fibroblasts.

Moreover, 3F52, but not Novus 19400002, detected the immunoreactive GLP-1R by immunohistochemistry in intestinal sections containing human Brunner glands (Fig. 7A and 7B and data not shown) and in human pancreatic islets (Fig. 7C and 7D).

Analysis of multiple histological sections from the ventricles of 35 different human hearts failed to reveal individual GLP-1R–immunopositive cells using the 3F52 GLP-1R antibody (representative staining depicted in Fig. 7E–7P). We next examined whether *GLP1R* mRNA transcripts

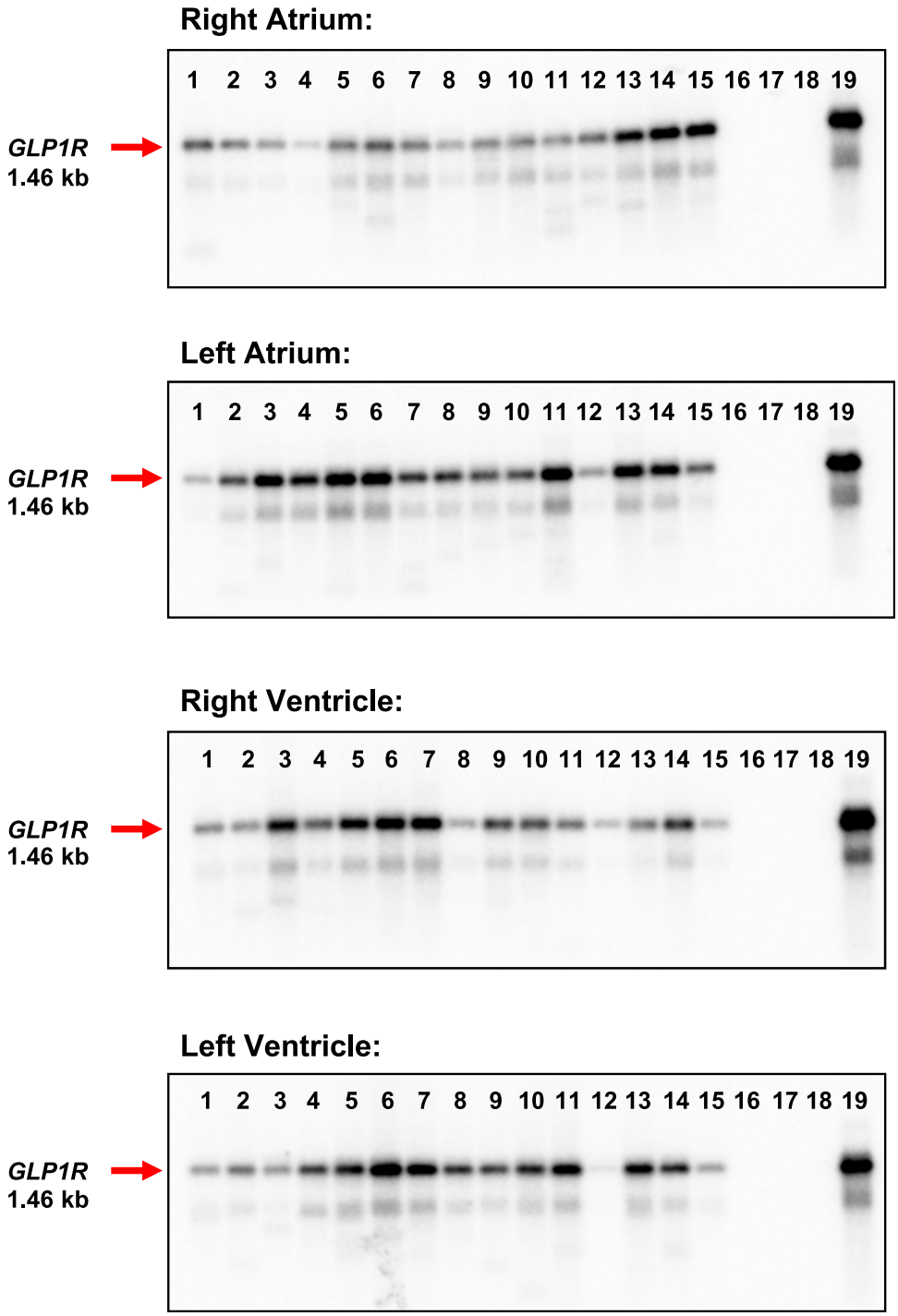

Figure 2. A full-length *GLP1R* is expressed in all four chambers of the human heart. RT-PCR analysis and Southern blotting using an internal *GLP1R*-specific oligonucleotide probe detected a 1.46-kb *GLP1R* mRNA transcript (red arrow) in the left and right atria and in the left and right ventricle of all 15 human hearts. Lanes 1 to 15, human heart samples; lane 16, negative control (H_2O); lane 17, human cardiac fibroblasts; lane 18, human adipose tissue; lane 19, human pancreas (positive control). See Supplemental Table 1 for a description of the human heart samples.

could be detected within a specific cardiac cellular subtype, such as cardiac myocytes, blood vessels, fibroblasts, and epicardial fat. We first assessed the sensitivity of our human *GLP1R* ISH assay, which detected *GLP1R* expression within human pancreatic islets and Brunner glands (Fig. 8A and 8B). Moreover, the suitability of representative human heart sections for ISH analyses was illustrated by detection of PECAM1 within endothelial cells (Fig. 8C). Although *GLP1R*+ cells were detected by ISH in atrial tissue from the human sinoatrial node region (Fig. 9M), we were unable to identify individual *GLP1R*+ cells, including analysis of cardiac myocytes, fat, and blood vessels, in histological sections from 35 human ventricular

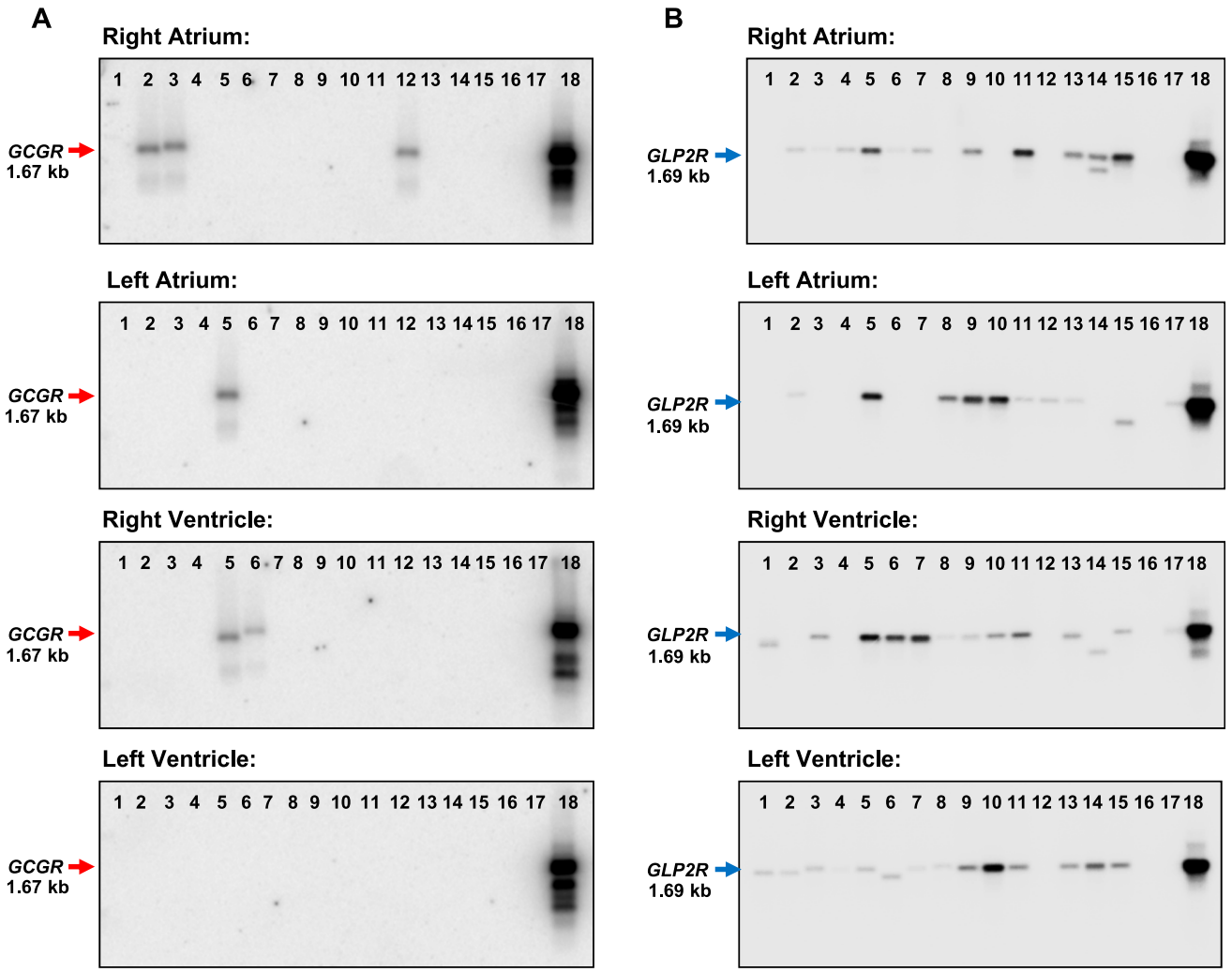

Figure 3. *GCGR* and *GLP2R* mRNA expression in the human heart. RT-PCR analysis and Southern blotting using an internal oligonucleotide probe specific to each receptor RNA detected a 1.67-kb *GCGR* mRNA transcript (red arrow, left panels) in a small number of human hearts and a 1.69-kb *GLP2R* mRNA transcript (blue arrow, right panels) in all four cardiac chambers of several human heart samples. Lanes 1 to 15, human heart samples; lane 16, negative control (H_2O); lane 17, human cardiac fibroblasts (*GCGR* blots) or human adipose tissue (*GLP2R* blots); lane 18 (positive control), human liver (*GCGR* blots) or human colon (*GLP2R* blots). See Supplemental Table 1 for a description of the human heart samples.

samples by ISH (representative ISH results are shown in Fig. 9A–9L).

Discussion

The demonstration that native GLP-1 and GLP-1R agonists rapidly improve ventricular function in the normoxic and ischemic human heart (14, 15, 33), taken together with the previous detection of GLP-1Rs within human and nonhuman primate atrial tissue (23), implicates one or more direct roles for GLP-1R signaling in the human heart. Despite reports from cardiovascular outcome studies that the GLP-1R agonist liraglutide reduced the rates of cardiovascular death and nonfatal myocardial infarction in human subjects with T2D (21), the precise localization and relative expression of the GLP-1R within human cardiac tissue has not been systematically examined. Here we report the results of our studies examining not only *GLP1R* but also *GCGR* and *GLP2R* expression in all four chambers of the human heart.

Consistent with results presented in publicly available transcriptomic analyses (34), levels of *GLP1R* mRNA transcripts in human heart were comparable to those detected in RNA from human pancreas. Moreover, our data extend these results by revealing detectable *GLP1R* mRNA transcripts in all four cardiac chambers. Levels of *GLP1R* mRNA transcripts were lowest in RNA isolated from the left ventricle. Despite functional expression of the *Gcgr* in mouse heart, including right and left ventricle (30), we were unable to detect evidence for *GCGR* expression, the receptor mediating the effects of glucagon, in most of the hearts examined, including in adult human left ventricle. In contrast, mRNA transcripts for the structurally related *GLP2R*, the receptor

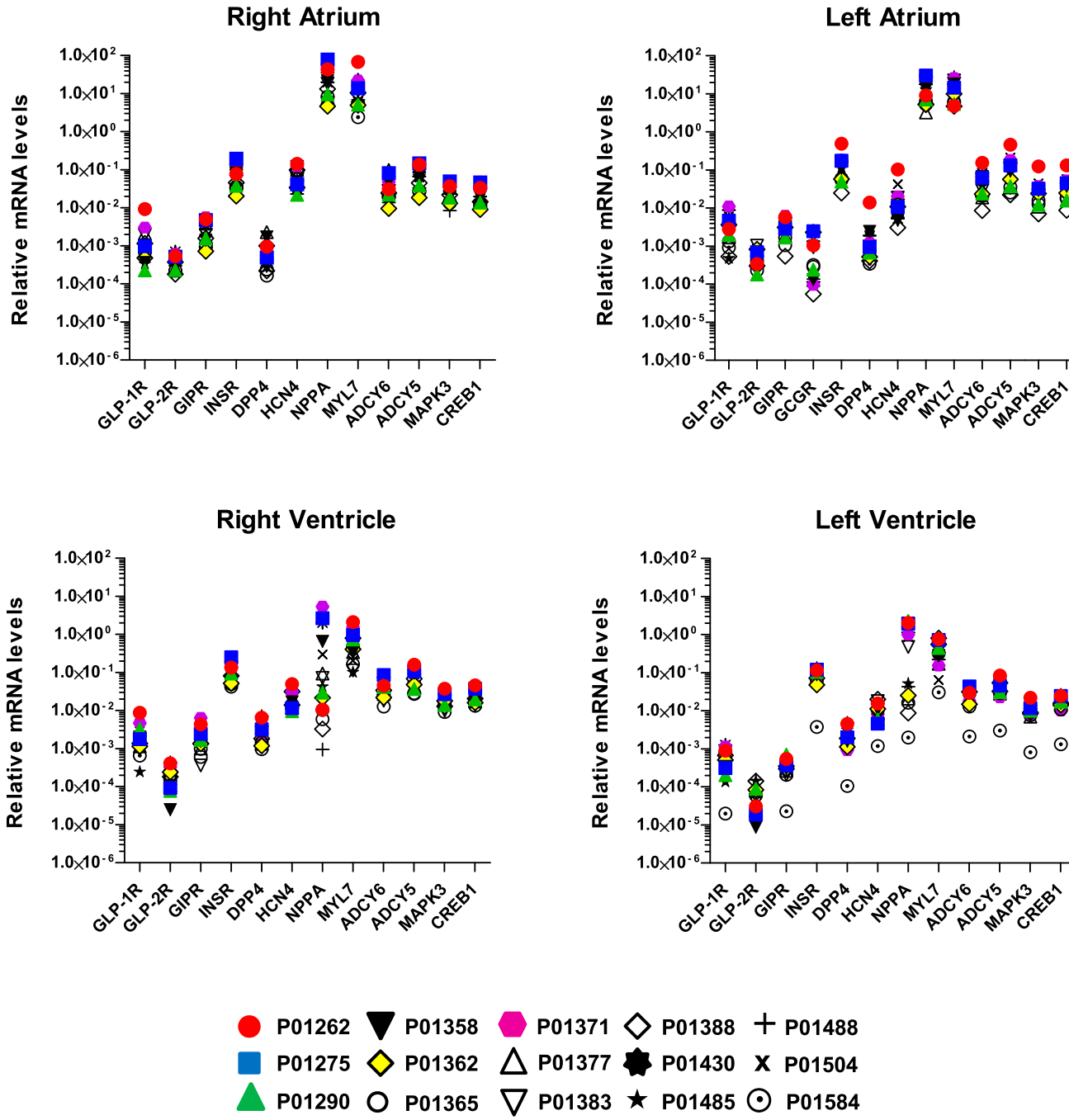

Figure 4. Quantitative gene expression profiles in all four chambers of the human heart. The mRNA levels of 12 or 13 different genes were measured in the four heart chambers from all 15 subjects via qPCR analysis. Data were normalized to β-actin. Each symbol represents analysis of RNA from an individual human heart (see Supplemental Table 1).

transducing actions of GLP-2, were extremely low yet were detected in most ventricular samples from the human hearts studied.

The relatively similar abundance of *GLP1R* mRNA transcripts in all four chambers of the human heart reported here differs from the expression pattern previously reported in rats and mice. *Glp1r* expression has been detected predominantly in the atria of normotensive and hypertensive mice (30, 35), whereas *Glp1r* mRNA transcripts were expressed at very low levels in the mouse cardiac ventricles (30) and were not detected in rat ventricular tissue or ventricular cardiomyocytes (36). In contrast, *GLP1R* mRNA transcripts were detected at comparable levels in ventricular and atrial RNA from the human heart in this study, although levels were generally lower in RNA isolated from the left ventricle. Species specificity of *GLP1R* expression has also been demonstrated in the thyroid, with detectable expression of the *Glp1r* in the rodent C-cell and thyroid (37) and extremely low-level or

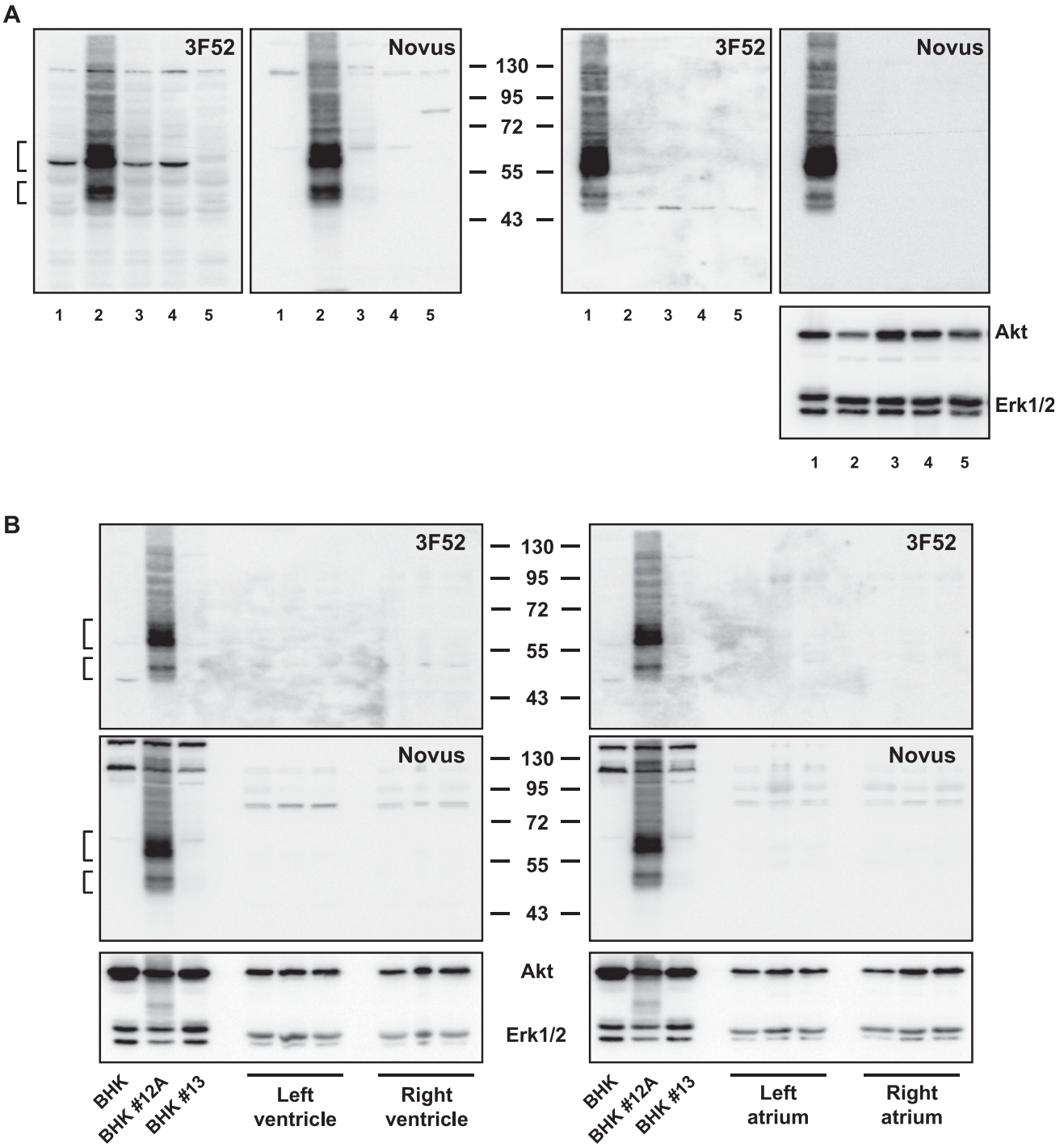

Figure 5. (A) Western blot analysis of whole cell or human islet tissue extracts using GLP-1R antibodies against the human GLP-1R, Mab 3F52, or polyclonal Novus. Molecular mass standards (kDa) are indicated in the center. Blots on the left contain whole cell extracts from BHK pcDNA3 (lane 1), BHK clone #12A (lane 2), BHK clone #13 (lane 3), BHK clone #27 (lane 4), and CFPAC-1 cells (lane 5). Blots on the right contain whole cell extracts from BHK clone #12A (lane 1) and whole tissue extracts from human islets #167 (lane 2), #177 (lane 3), #186 (lane 4), and 199 (lane 5). The blot on the far right was immunoblotted with Akt and Erk1/2 antibodies as loading controls. (B) Western blot analysis of whole cell or human cardiac tissue extracts analyzed using the indicated GLP-1R antibody. Molecular mass standards (kDa) are indicated in the center. Immunoblotting with Akt and Erk1/2 antibodies (bottom panels) was used as loading controls. In (A) and (B), the brackets indicate predicted migration positions of immunoreactive GLP-1R protein.

undetectable *GLP1R* expression in the nonneoplastic human thyroid (27). Furthermore, our current analyses of atrial *GLP1R* expression did not routinely include RNA isolated from the human sinoatrial node, a region of the human heart that expresses abundant *GLP1R* mRNA and protein (23). Hence, our findings reinforce the importance of considering species-specific differences and variation in receptor expression

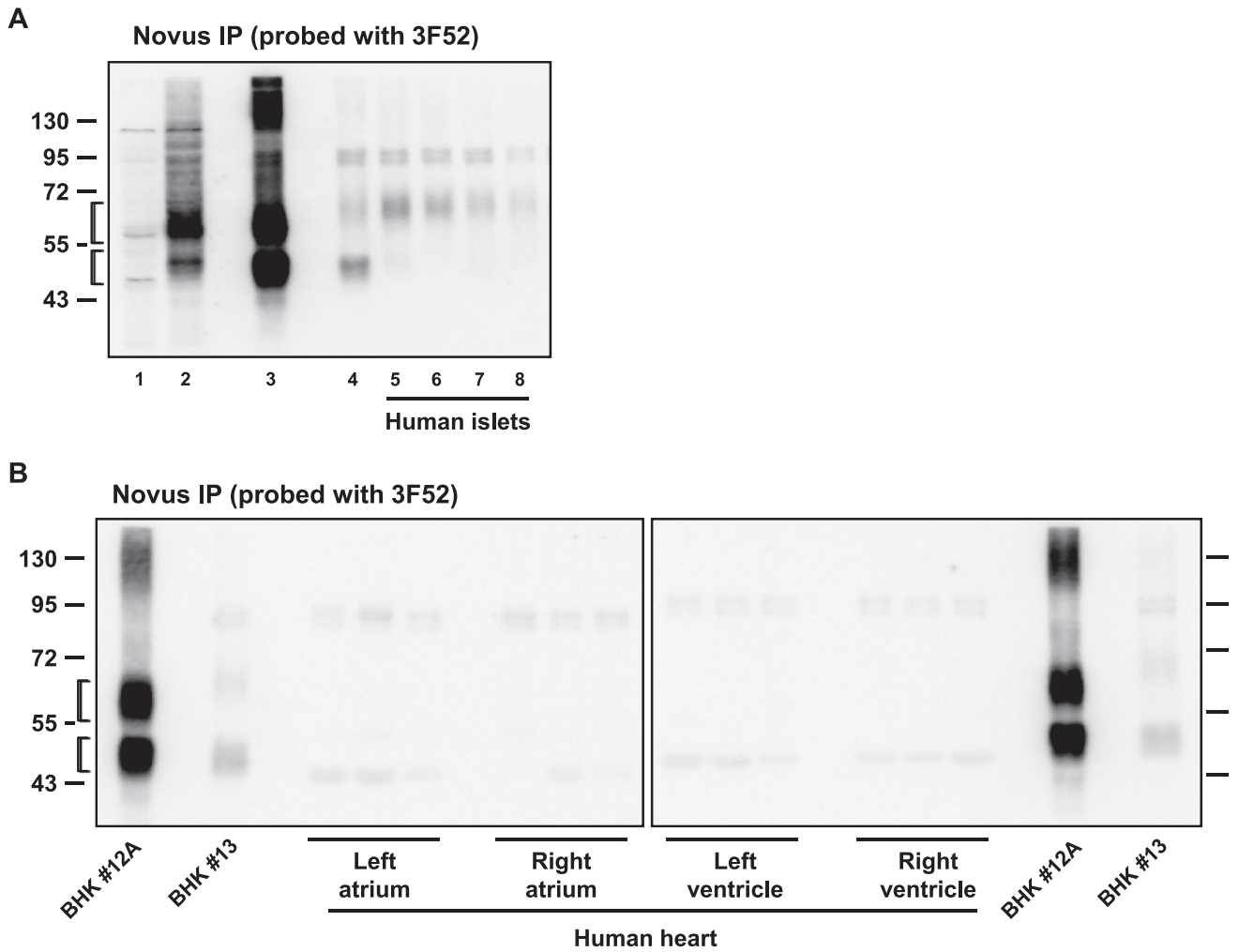

Figure 6. (A) Whole cell/tissue extracts after immunoprecipitation (IP) and Western blotting with the indicated GLP-1R antibodies. Lanes 1 and 2 are whole cell extracts, and lanes 3 through 8 correspond to immunoprecipitated samples. Lane 1, BHK pcDNA3; lane 2, BHK clone #12A; lane 3, BHK clone #12A; lane 4, BHK clone #13; lane 5, human islet #167; lane 6, human islet #177; lane 7, human islet #186; and lane 8, human islet #199. Molecular mass standards (kDa) are indicated on the left. (B) Whole tissue extracts from human heart chamber samples after IP and Western blotting with the indicated GLP-1R antibodies. Molecular mass standards (kDa) are indicated on the far left and right of the blots. In (A) and (B), the brackets indicate predicted migration positions of immunoreactive GLP-1R protein.

arising from differential RNA sampling when interpreting relative GLP-1R expression data from preclinical and human studies.

GLP-1R agonists acutely increased myocardial blood flow in human subjects with diabetes without coronary artery disease (38), and infusion of native GLP-1 rapidly augmented myocardial blood flow in human subjects without diabetes (39). Moreover, an immunoreactive GLP-1R has been reported in studies of human coronary artery endothelial cells and in rat kidney vascular smooth muscle cells (40, 41). Similarly, GLP-1 directly induced elastic fiber formation in studies of human cardiac fibroblasts (42). In contrast to reports of vascular *Glp1r*-directed reporter expression in murine coronary arteries (43), we were unable to detect expression of canonical *GLP1R* mRNA transcripts in RNA from human coronary artery endothelial cells, human coronary artery vascular smooth muscle cells, or human cardiac fibroblasts. Hence, the extent to which these noncardiomyocyte cell types may, under some circumstances, express the GLP-1R within regions of the human heart requires further confirmation.

In the course of assessing the suitability of two different antisera for detection of the cardiac GLP-1R, we were unable to detect immunoreactive GLP-1R by conventional Western blotting using extracts from several different preparations of human islets. In contrast, both antisera readily detected the human GLP-1R in Western blot analysis of hGLP-1R–transfected fibroblasts. Although a putative endogenous GLP-1R protein has been detected in multiple cell lines and tissue extracts, including islets and heart, by many groups using Western blot analysis (44–47), the sensitivity and specificity of the majority of antisera used for these studies has not been

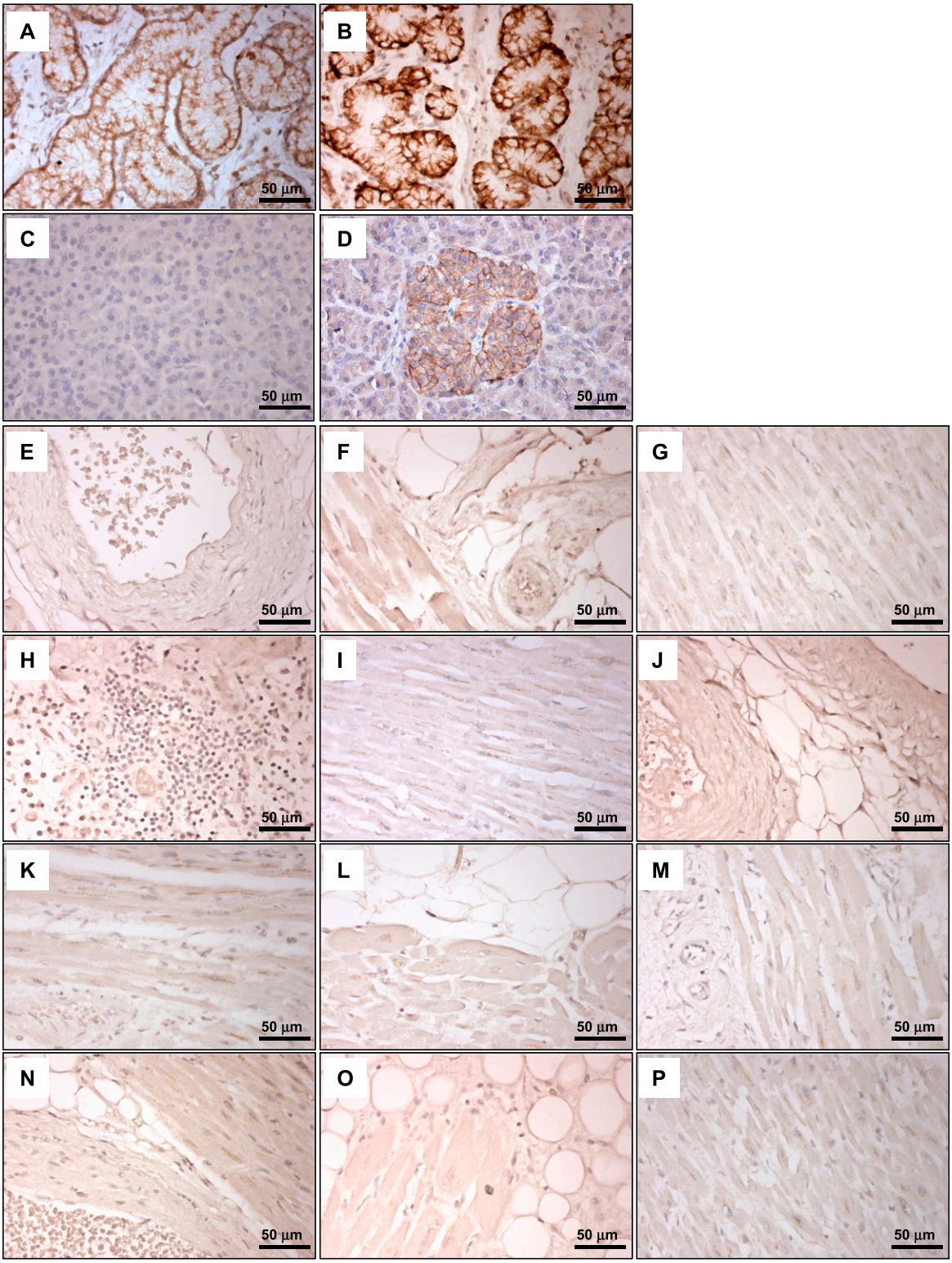

Figure 7. GLP-1R immunohistochemistry with Mab 3F52 does not detect GLP-1R–immunoreactive cells in human ventricular tissue. Immunostaining of hyperplastic human Brunner glands with isotype control antibody (A) and GLP-1R Mab 3F52 (B). The Novus 19400002 antibody did not reliably detect GLP-1R–immunopositive cells in sections containing human Brunner glands (data not shown). Immunostaining of human pancreas with isotype control antibody (C) and GLP-1R Mab 3F52 (D). (E–P) GLP-1R Mab 3F52 immunostaining of human cardiac tissues from individual subjects [(E) 193856; (F) 61704; (G) 70150; (H) 70847; (I) 81858; (J) 164011; (K) 116603; (L) 166469; (M) 179268; (N) 171263; (O) 182651; (P), 52411; see Supplemental Table 2].

uniformly verified (24, 25, 31). Because the β-cell is among the cell types exhibiting the most robust expression of the endogenous GLP-1R, our inability to detect expression of the endogenous GLP-1R in conventional Western blotting of human islets provides a further note of caution regarding the accuracy of multiple reports of

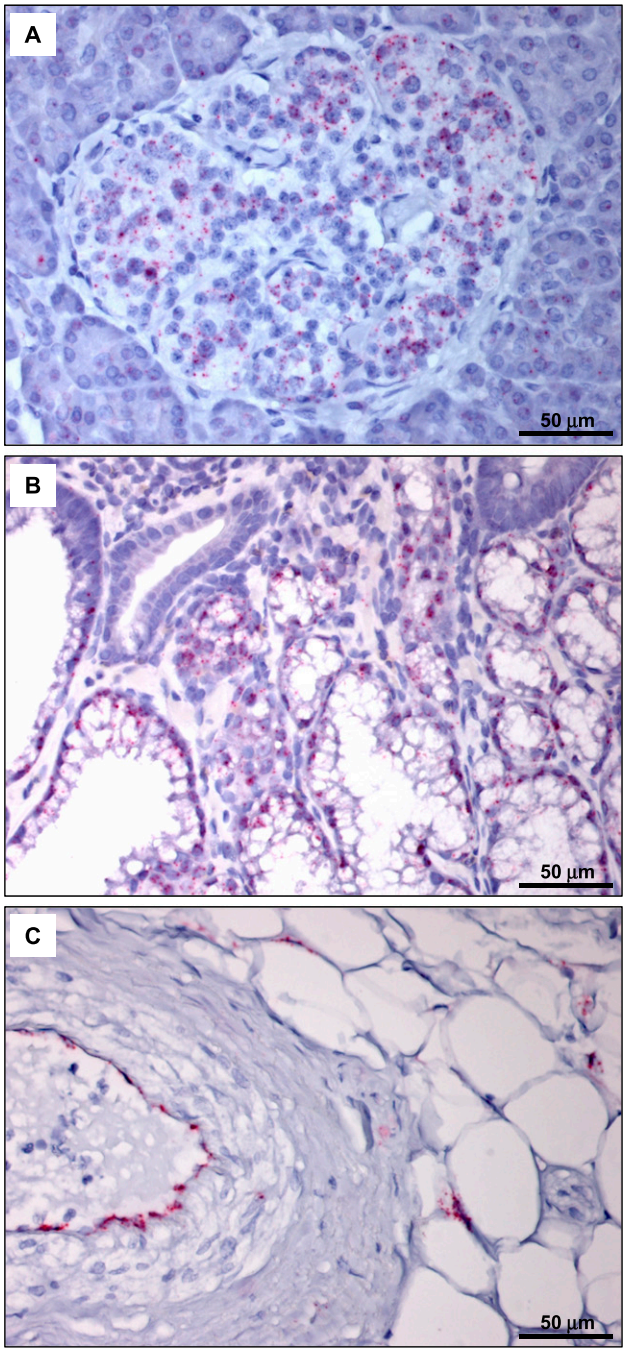

Figure 8. ISH analysis detects *GLP1R* mRNA expression in human pancreas (A) and hyperplastic human Brunner glands (B). *GLP1R*-positive cells are indicated by punctate pink staining. (C) Detection of *PECAM1* mRNA expression in human heart (case 164011; Supplemental Table 2) verifies the quality of the tissue and the integrity of the mRNA.

GLP-1R protein expression using Western blotting and native tissue extracts.

In summary, we detected low-level expression of the full-length *GLP1R* mRNA transcript in all four chambers of the human heart. In contrast, mRNA transcripts encoding the structurally related receptors for peptides coencoded by the same proglucagon precursor (*e.g.*, glucagon and GLP-2) were not detected (*GCGR*) or were detected at even lower levels in a less uniform manner (*GLP2R*). Despite a previous report detecting *GLP1R* mRNA transcripts in human ventricular myocytes by RT-PCR (22), a combination of ISH and immunocytochemistry was not successful in localizing GLP-1R expression to individual cell types within the dozens of human heart sections studied here.

Caveats and limitations

These studies have several limitations. It may be important that our PCR analyses were carried out on RNA obtained from cardiac tissue subjected to cardioplegia followed by rapid freezing prior to RNA preparation. In contrast, although the human heart samples we obtained for histological analysis from the Rapid Autopsy Program were fixed relatively quickly (often within hours) to minimize degradation of RNA and protein, we cannot exclude the possibility that failure to localize *GLP1R* RNA and protein within specific cells in the heart may reflect instability of the RNA transcript or protein, compromising detection. Furthermore, although we analyzed the tissue sections by immunohistochemistry or ISH within several days to 2 weeks after generation of the slides, it is possible that the sensitivity of *GLP1R*/GLP-1R detection falls off rapidly over time in paraffin sections, further hampering *GLP1R* transcript or GLP-1R protein localization.

It seems likely, based on available murine data (43), that the cardiac GLP-1R may be expressed at very low levels in scattered cells, perhaps within a subset of nerves or blood vessels, including endothelial cells (48), or adjacent to valves not contained within the several dozen heart sections examined here. A complementary possibility limiting detection of the GLP-1R protein is that *GLP1R* mRNA transcripts may not be uniformly translated into a detectable GLP-1R protein in all cardiac cells expressing the *GLP1R* mRNA. Alternatively, cardiac-specific posttranslational modifications of the GLP-1R may compromise detection of the immunoreactive protein using the 3F52 antibody. Furthermore, the relative level of *GLP1R* expression within individual cardiac cells may be very low, precluding definitive identification by ISH. Hence, further studies, perhaps using cell enrichment techniques, ideally from heart samples rapidly obtained from well-preserved hearts, may be required to enable more sensitive and precise identification of the cellular sites of GLP-1R expression in human cardiac tissue. Nevertheless, our studies highlight the consistent detection of low-level *GLP1R* expression within human ventricular tissue, findings with potential relevance for understanding how GLP-1R agonists directly affect cardiac function *in vivo*.

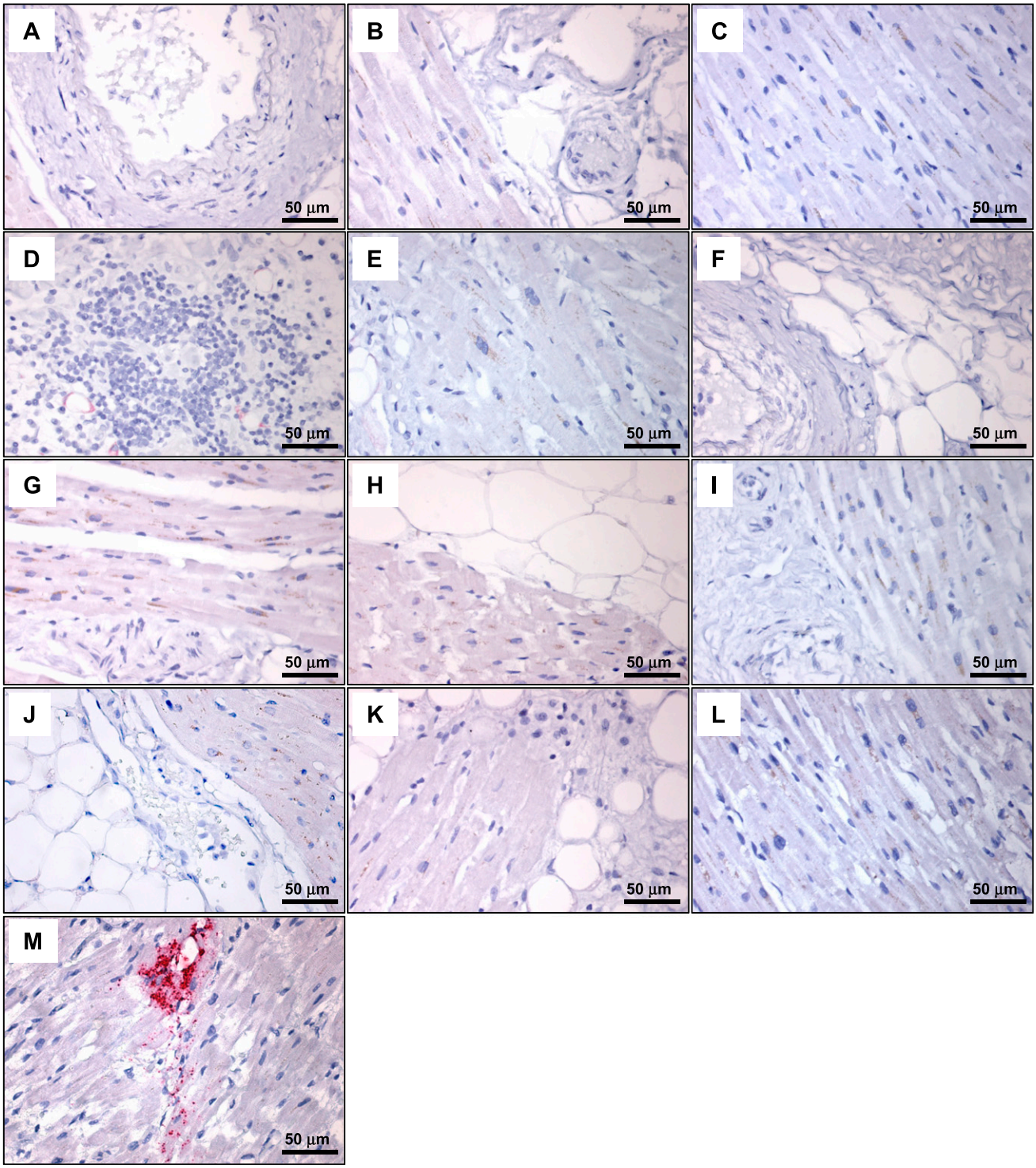

Figure 9. ISH analysis of *GLP1R* mRNA expression detects *GLP1R*-positive cells in human atrial tissue from the sinoatrial node region but not in human ventricular tissue. (A–L) ISH analysis of *GLP1R* mRNA expression in cardiac tissues from the following cases (see Supplemental Table 2): (A) 193856; (B) 61704; (C) 70150; (D) 70847; (E) 81858; (F) 164011; (G) 116603; (H) 166469; (I) 179268; (J) 171263; (K) 182651; (L) 52411. (M) ISH analysis of *GLP1R* mRNA expression in tissue from human sinoatrial node region.

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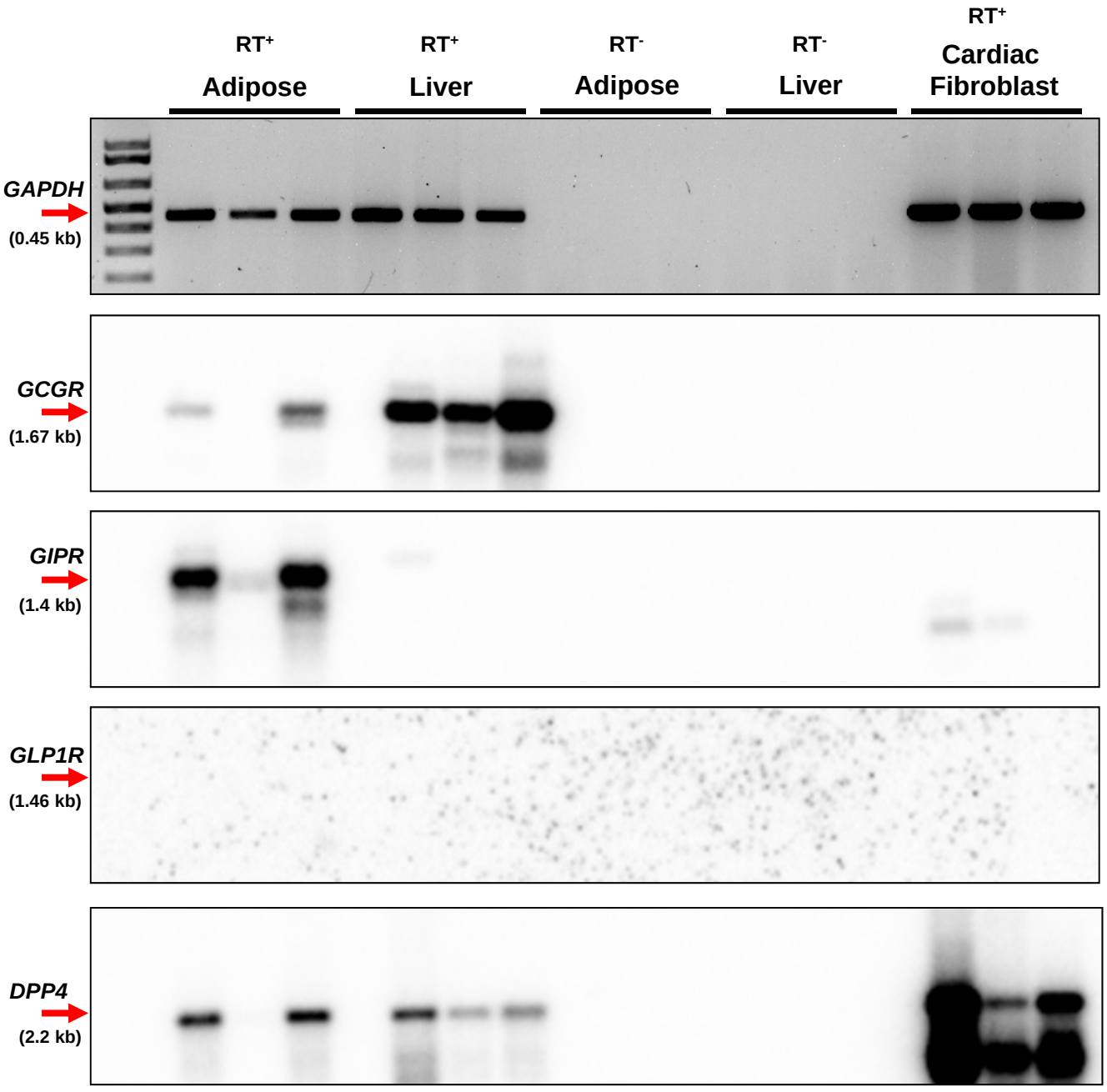
Supplementary Figure 1



Supplementary Table 1. Clinical characteristics of human heart tissue donors

Sample	Patient ID	Tissue Source	CHF Etiology	Age (years)	Weight (kg)	BMI (kg/m^2)	HW (g)	LV Mass (g)	HW Index	Afib	VT/VF	Diabetes	Hypertension	LVEF	Creatinine (mg/dl)
1	P01262	C	ID-CMP	42	115	35.5	528	N/A	220	Y	N	N	N	0.15	1
2	P01275	C	ID-CMP	62	83	24.8	662	N/A	315	Y	N	N	N	0.1	1.25
3	P01290	C	ID-CMP	65	68	21	624	433	347	N	N	N	N	0.1	1.27
4	P01358	C	ID-CMP	42	89	23.4	744	392	338	Y	Y	Y	Y	0.1	1.1
5	P01362	NF	NF	51	91	30.4	286	156	136	U	U	Y	U	0.55	0.8
6	P01365	NF	NF	69	67	20	377	176	209	N	N	N	Y	U	1.08
7	P01371	C	ID-CMP	54	60	20	434	244	255	N	Y	N	N	0.15	1.29
8	P01377	NF	NF	77	78	27	495	181	261	Y	N	N	Y	U	0.8
9	P01383	NF	NF	59	132	43.1	498	234	199	N	N	Y	Y	0.6	0.88
10	P01388	NF	NF	51	121	36.1	516	265	206	N	N	N	N	0.55	1.5
11	P01430	C	ID-CMP	62	86	22.6	730	395	332	Y	N	N	N	0.12	1.08
12	P01485	NF	NF	78	92	29.9	448	234	213	N	N	N	Y	U	U
13	P01488	NF	NF	81	78	27	402	218	212	N	N	N	Y	U	1.6
14	P01504	C	ID-CMP	49	76	26.3	424	269	223	N	N	Y	Y	0.18	0.69
15	P01584	NF	NF	22	67	20.7	299	182	166	N	N	N	N	0.55	1

All cardiac tissue samples are from male, Caucasian patients. Afib, atrial fibrillation; BMI, body mass index; C, cardiomyopathy; CHF, chronic heart failure; HW; heart weight; ID-CMP, idiopathic cardiomyopathy; LV, left ventricle; LVEF, left ventricular ejection fraction; N, no; NF, non-failing; VT/VF, ventricular tachycardia/ventricular fibrillation; U, unknown; Y, yes. Modified from Ussher, JR et al Reference 26

Supplementary Table 2. University Health Network Rapid Autopsy Program: Human heart samples

Case #	Tissue Site	Procedure
52411	Cardiac ventricle, right and septum	Autopsy
61704	Cardiac ventricle, right	Autopsy
69184	Cardiac ventricle, right	Autopsy
70150	Cardiac ventricle, right	Autopsy
70847	Coronary artery	Autopsy
73434	Cardiac ventricle, right	Autopsy
75924	Cardiac ventricle, left	Autopsy
76623	Cardiac ventricle, left	Autopsy
77566 N	Cardiac ventricle, left	Autopsy
81342	Cardiac ventricle, left	Autopsy
81346	Cardiac ventricle, left	Autopsy
81858	Cardiac ventricle, left	Autopsy
81862	Cardiac ventricle, left	Autopsy
82711	Cardiac ventricle, left and right	Autopsy
84855	Cardiac ventricle, left with vessels	Autopsy
116603	Left and right septum	Autopsy
137710	Cardiac ventricle, left with leukemic infiltrate	Autopsy
159096	Cardiac ventricle, left	Autopsy
166469	Cardiac ventricle, left with epicardial fat	Autopsy
179268	Cardiac ventricle, left	Autopsy
179338	Cardiac ventricle, left with lymphoma	Autopsy
193847	Cardiac ventricle, right and coronary artery	Autopsy
193856	Cardiac ventricle, left with small coronary arteries	Autopsy
152781 N	Cardiac ventricle, left	Autopsy
164007 N	Cardiac ventricle, right	Autopsy
164011 N	Cardiac ventricle, left with epicardial vessels	Autopsy
171263 N	Cardiac ventricle, right with inflammation	Autopsy
171267 N	Cardiac ventricle, left and septum	Autopsy
176225 N	Cardiac ventricle, right with small vessels and fat	Autopsy
180806 A	Heart	Autopsy
180820 N	Cardiac ventricle, left	Autopsy
180827 N	Cardiac ventricle, right	Autopsy
182647 N	Cardiac ventricle, left with infarct scar and small arteries	Autopsy
182651 N	Heart	Autopsy
212080 N	Cardiac ventricle, left with small amount of tumour	Autopsy

Supplemental Table 3. Alberta Diabetes Institute Islet Core-supplied human islet donor information

Donor #	Age (years)	Gender	BMI	HbA1c
R167	84	female	21.10	NA
R177	28	male	23.39	NA
R186	67	male	28.06	5.6
R199	56	male	18.32	5.3
R200	65	male	27.13	5.1

BMI, body mass index; HbA1c, hemoglobin A1c.

Supplementary Table 4. qPCR Primers for TaqMan gene expression

Gene	Description	Assay ID
ACTB	Beta actin	Hs99999903_m1
GLP1R Exon 3-4	Glucagon-like peptide-1 receptor	Hs00157705_m1
GLP2R	Glucagon-like peptide-2 receptor	Hs00173688_m1
GIPR	Gastric inhibitory polypeptide receptor	Hs00609210_m1
GCGR	Glucagon receptor	Hs00164710_m1
INSR	Insulin receptor	Hs00961560_m1
GPI	Glucose-6-phosphate isomerase	Hs 00976715_m1
DPP4	Dipeptidyl Peptidase 4	Hs00897391_m1
HCN4	Hyperpolarization activated cyclic nucleotide gated potassium channel 4	Hs00975492_m1
NPPA	Natriuretic peptide A	Hs00383230_g1
MYL7	Myosin light chain 7	Hs01085598_g1
ADCY6	Adenylate cyclase 6	Hs00209600_m1
ADCY5	Adenylate cyclase 5	Hs00766287_m1
MAPK3	Mitogen-activated protein kinase 3	Hs00385075_m1
CREB1	cAMP response element binding protein 1	Hs00231713_m1
CHMP2A	Charged multivesicular body protein 2A	Hs00205423_m1
EMC7	ER membrane protein complex subunit 7	Hs00220077_m1
PSMB4	Proteasome subunit, beta type 4	Hs00160598_m1
VIM	Vimentin	Hs00958111_m1
ICAM1	Intercellular adhesion molecule 1	Hs00164932_m1
TEK (Tie2)	TEK receptor tyrosine kinase	Hs00945150_m1
TAGLN	Transgellin	Hs01038777_g1
SMTN	Smoothelin	Hs01022255_g1
PTPRC (CD45)	Protein tyrosine phosphatase, receptor type C	Hs04189704_m1
THY1 (CD90)	Thy-1 cell surface antigen	Hs00174816_m1
ENG (CD105)	Endoglin	Hs00923996_m1

All primers are from Thermo-Fisher Scientific.