Supplemental Figure 1. Transfected cells and Western blotting.
A. IHC staining with MAb 3F52 of human GLP-1R expressed in BHK cells. Two different IHC protocols (refer to Research Design and Methods) were tested on high, low and non-transfected cells.
B. Western blotting experiment with MAb clone 3F52 (1:500), detected with goat-anti-mouse (1:5000). Cell pellets were lysed in TissueLyser II (Qiagen), plasma membranes were treated with PNGaseF enzyme to reduce N-linked glycosylation prior to boiling in SDS loading buffer with DTT. Lane 1: HEK293 cells transfected with human GLP-1R. Lane 2: HEK293 cells transfected with rabbit GLP-1R. Note that rabbit GLP-1R is not detected, in agreement with the predicted species reactivity of MAb 3F52.

Supplemental Figure 2. Monkey kidney.
GLP-1R IHC with antibody MAb 3F52 on frozen (A–C), and paraffin-embedded rhesus monkey kidney (M–P), and in situ ligand binding with 125I-GLP-1 on frozen section from rhesus monkey kidney cortex (G–I). D–F are images from section adjacent to A and incubated with isotype control antibody at same concentration. J–L are images from section adjacent to G and incubated with 125I-GLP-1 plus an excess of unlabelled GLP-1. B, E, H and K are higher magnification of dotted-line box areas in A, D, G and J, respectively. C, F, I and L are higher magnification of solid-line box areas in A, D, G and J, respectively.
In A–C GLP-1R immunoreactivity can be seen to be mainly membrane-associated on individual smooth muscle cells in the wall of preglomerular arcuate artery (B), interlobular artery (large vessel in C) and arterioles (three small vessels in C).
In G–I, a strong autoradiography signal is seen completely overlapping with the GLP-1R IHC signal. With both IHC and ISLB, both glomeruli and tubules are devoid of signal.
M–P show examples of GLP-1R (green)/SMA (red) double immuno-fluorescence, In M, a GLP-1R/SMA double positive arteriole is seen budding from a GLP-1R negative/SMA positive artery.
N–P show examples of GLP-1R/SMA double positive arterioles immediately adjacent to glomeruli (circles). Scalebars: 1mm (A,D,G,J); 100µm (all other).

Supplemental Figure 3. Monkey pancreas.
A–F: Double-fluorescence IHC on adjacent sections of a rhesus monkey islet of Langerhans for GLP-1R/insulin (A), and GLP-1R/glucagon (C–F). In B, an intense and membrane-associated staining (green) for GLP-1R is seen in endocrine cells, and these cells show a complete overlap with insulin producing cells (red, A). In C, glucagon producing cells (red) are seen adjacent to GLP-1R positive cells (green). D–F are higher magnification of area in C. GLP-1R staining of membranes surrounding alpha-cells (* in E) likely represents cytoplasmic extensions of beta-cells. This interpretation is supported by the absence of GLP-1R staining of part of alpha cell membranes (arrows in E). D shows glucagon producing cells in red, and F is a merged image of D and E.
G–H: Montage of low magnification overview of GLP-1R immunofluorescence signal (green) in pancreas samples containing main duct areas from 6 diabetic (G) and 6 normal (H) rhesus monkeys. Note absence of immunopositive cells in the main duct (askerisks) of all samples, whereas islets located in the vicinity of ductal structures are positive for GLP-1R (green). All sections have been counterstained with DAPI to highlight cell nuclei.
I: ISLB with 0,3nM 125I-GLP-1 in monkey pancreas. Quantitation of silvergrains was performed using automated image analysis as described previously (9), and the counts are presented as silvergrains pr. area in islets and acinar cells, respectively. The ISLB signal in both beta-cells and acinar cells can be fully competed with both 100nM unlabelled GLP-1 and 50ug/ml MAb clone 3F52, but not with 50ug/ml anti-LAGH (isotype control antibody). This is consistent with the fact
that 3F52 neutralizes the binding of GLP-1 to primate GLP-1R (S. Reedtz-Runge, unpublished data). These quantitative data indicate the relative GLP-1 receptor density per area in islets vs. acini to be ~5 in monkey.

**Supplemental Figure 4. Human pancreas.**
A-V: Montages of micrographs of GLP-1R immunostaining in samples containing different duct tree compartments from a total of 22 human pancreata (listed in Supplementary Table 1). Note absence of GLP-1R positive ductal epithelial cells in all samples. All sections were scanned in a Nanozoomer 2.0HT slide scanner (Hamamatsu, Denmark), digital images were analysed and representative areas were used for the figures.

Abbreviations:
LI= Islets of Langerhans
ID= Intercalated Duct
IDt=Intralobular Duct
IDe= Interlobular excretory Duct
PD= Pancreatic Duct
PDG= Pancreatic Duct-like Glands
P= Ductal proliferative response
W: Example to demonstrate that rare GLP-1R positive cells seen in ducts or ductules are endocrine insulin positive cells.

**Supplemental Figure 5. Human heart.**
In A, the SAN region from a paraffin-embedded sample of a normal human heart is seen as mainly blue Trichrome stained area (center), with adjacent non-SAN myocyte areas staining mainly red (upper and lower left corner). B and C are from same area as A on near-adjacent sections immunostained for GLP-1R and with isotype control antibody, respectively. Askerisk marks SAN artery. D, E and F are higher magnification micrographs from dotted-line box areas in the central area of the SAN in A, B and C, respectively. G, H and I are higher magnification micrographs from solid-line box areas within non-SAN myocyte area in A, B and C, respectively. Note that only SAN myocytes express GLP-1R. Scalebars: 3mm (A-C); 300µm (D-I).

**Supplemental Figure 6. Monkey gastrointestinal tract.**
In the monkey stomach, low GLP-1R expression is seen in smooth muscle cells in the muscularis externa (A) (M=Mucosa, MM=Muscularis Mucosa, SM=Submucosa, ME=Muscularis Externa). B is high magnification micrograph of boxed area in A. In C, a subset of parietal cells in the monkey stomach epithelium display GLP-1R immunoreactivity with MAb 3F52. D is from same region as C on adjacent section incubated with isotype control antibody.
E-I is from a monkey small intestine and highlights GLP-1R immunoreactivity in neurons in the myenteric plexus. In a HE section of monkey ileum (E) (CSM=Circular Smooth Muscle Layer, LSM=Longitudinal Smooth Muscle Layer), a magnification of the boxed area highlights a cluster of myenteric plexus neurons (F). The same area from an adjacent section stained with MAb 3F52 (G) display GLP-1R immunoreactivity in a cluster of cells that can clearly be identified as the same myenteric plexus neurons. H shows GLP-1R immunoreactive myenteric plexus neurons in monkey jejunum clearly identified as such on HE of near-adjacent section (I).
In J-K, prominent GLP-1R expression is seen at the baso-lateral membrane of Brunner’s gland epithelial cells in the monkey duodenum. K is higher magnification of boxed area in J. L is in situ ligand binding with $^{125}$I-GLP-1 on frozen section from rhesus monkey duodenum, with clear signal
in Brunner’s gland epithelial cells that can be displaced by incubation with $^{125}$I-GLP-1 plus an excess of unlabelled GLP-1 (M).
Supplemental Table 1: Human pancreas tissue blocks used for GLP-1R IHC.
All samples were normal appearing tissue from surgical resection specimens from patients operated for condition designated as “Diagnosis” column in the table, and HE sections cut from each tissue block was histologically evaluated by RKK (“Histology” column in table).

<table>
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<th>#</th>
<th>ID</th>
<th>DM/NDM*</th>
<th>Diagnosis</th>
<th>Histology</th>
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<tr>
<td>1</td>
<td>736-10</td>
<td>T1DM</td>
<td>Pancreas adenocarcinoma, ductal adenocarcinoma</td>
<td>Moderate to marked fibrosis and infiltration of inflammatory cells</td>
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<td>Pancreas, adenoma of ampulla of Vater</td>
<td>Mild fibrosis</td>
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<td>Areas with neoplasia</td>
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<tr>
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<td>Areas with neoplasia</td>
</tr>
<tr>
<td>5</td>
<td>740-10</td>
<td>T2DM</td>
<td>Mucinous cystadenoma of pancreas</td>
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<tr>
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<td>T2DM</td>
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<td>Mild infiltration of inflammatory cells</td>
</tr>
<tr>
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<td>T2DM</td>
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<td>Moderate to marked fibrosis, ductular hyperplasia and mucinous metaplasia of ductular epithelium</td>
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<tr>
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<td>Areas with neoplasia</td>
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</tbody>
</table>

*DM=Diabetic (Type-2 (T2DM) or Type-1 (T1DM)); NDM= Non-diabetic
Supplemental Figure 1
(A-B) – 1 page

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A

Human GLP-1R transfected cells (high)

Human GLP-1R transfected cells (low)

Untransfected cells (control)

DAB  Fluorescence

B

110 kDa ➔

50 kDa ➔

15 kDa ➔
Supplemental Figure 4 (A-W) – 23 pages

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T1DM
#736
Panc.
Adenoc.
Ductal

A
T2DM
#738
Panc.
Ductal
Adenoc.
Specimen
w. Neoplasia

C
T2DM
#739
Adenoc. of bile duct

Specimen w. Neoplasia
T2DM #740
Muc. cyst-adenoma of pancreas
Specimen w. Neoplasia
Non-diab.
#1146
Panc.
Adenoc.
Non-diab. #1150 Panc. Adenoma

K
Non-diab.
#1152
Histiocytoma
Non-diab. #1154
Panc. Ductal Adenoc.
Non-diab. #7222
Panc. Adenoc.
Specimen w. Neoplasia
Non-diab. #7428
Renal cancer w. panc. metast.
Non-diab.
#7574
Obs. Panc.
Adenoc.
Non-diab. #7885
Panc. Adenoc.