General Methods

Abbreviations

Abbreviations used for amino acids and designation of peptides follow the rules of the IUPAC-IUB Commission of Biochemical Nomenclature in J. Biol. Chem. 1972, 247, 977-983. The following additional abbreviations are used: Alloc, allyloxycarbonyl; AMBER, Asisted Model Building Energy Refinement; BCM, β-cell mass;Boc, tert-butyloxycarbonyl; BB, bromophenol blue; t-Bu, tert-butyl; CDI carbonyldiimidazolide; CH3CN, acetonitrile; DCM, dichloromethane; Cy5, Cyanine 5 dye (for structure see **Scheme 3**); DEAD, diethyl azodicarboxylate; DI, de-ionized; DIPEA, diisopropylethylamine; DMF, N,N-dimethylformamide; DIC, diisopropylcarbodiimide; DMEM, Dulbecco's Modified Eagle Medium; DOTA, 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid; Fmoc, fluorenylmethoxycarbonyl; FT-ICR, Fourier Transform-Ion Cyclotron Resonance; ESI-MS, Electrospray ionization-mass spectrometry; EDT, 1,2 ethanedithiol; Et2O, Diethyl ether;HCTU, O-[1-H-6-chloro-benzotriazol-1-yl)(dimethylamino) ethylene] uraniumhexafluorophosphateN-oxide; HOBt, N-hydroxybenzotriazole; HOCt, 6-chloro-1-hydroxybenzotriazole; hCCK2R, human cholecystokinin 2 receptor; hδ-OR, human delta-opioid receptor; hMC4R, human Melanocortin-4 receptor; htBVL, heterobivalent ligand; MMFF, 5-HT1FR, 5-Hydroxytryptamine 1F receptor; GLP-1R, glucagon-like peptide 1 receptor; Glb, Glibenclamide; Merck Molecular Force Field; MSH, melanocyte-stimulating hormone; MALDI, Matrix Assisted Laser Desorption Ionization-Time of Flight; Pbf, 2,2,4,6,7-pentamethyl-dihydrobenzofuran-5-sulfonyl; Pego, 19-amino-5-oxo-3,10,13,16-tetraoxa-6-azanonadecan-1-oic acid; SPPS, solid-phase peptide synthesis; SD, stochastic dynamics; RP-HPLC, reverse-phase high performance liquid chromatography; SUR-1, sulfonylurea 1 receptor; TA, thioanisole; TEA, triethylamineTHF, tetrahydrofuran; TIS, triisopropylsilane; TFA, trifluoroacetic acid; Trt, triphenylmethyl (trityl).

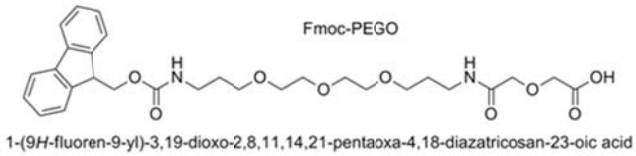

Figure S1. Structure of Fmoc-PEGO.

Cells and Culture Conditions

βTC3 cells were maintained under standard conditions (37°C, 5% CO2) and were grown in RPMI medium with 10% FBS and 1% Penicillin/Streptomycin. βTC3 from passages 20-30 in one lineage and passages from 42-52 from a second lineage were used. βTC3 cells express the sulfonylurea receptor at ~ 80,000 copies per cell and the GLP-1 receptor at ~ 250,000 copies/cell for a ratio of approximately 3:1 (see Figure S2 below). INS 832/3 cells were cultured under the same conditions with the addition of HEPES to the culture media.

Estimation of Receptor Number

Using the 96 well binding assay, the number of receptors per cell was calculated by evaluating the signal at saturation, obtained by fitting the data to a one-site specific non-linear regression equation in GraphPad Prism, and comparing these values to a Europium concentration calibration curve which yielded counts per molecule of ligand, as previously described. [1] From the data presented in Figure S2, there is an estiimate of 250,000 GLP-1R and 80,000 SUR1 receptors per βTC-3 cell.

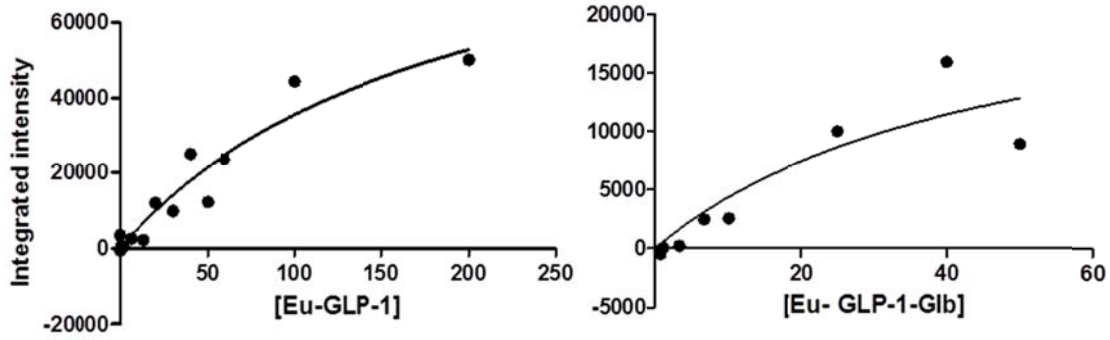

Figure S2. High throughput saturation binding analysis for estimation of receptor number. Saturation binding of Eu-GLP-1 was used to estimate the number of GLP-1R (Left). To obtain the number of SUR1 sites, the binding of the Eu-GLP-1/Glb was measured in the presence of 1 μM GLP-1 to block binding to the GLP-1R without affecting access to the SUR1 (Right graph). βTC3 cells were incubated in the presence of varying concentrations of Eu-labelled ligands for 1 hr, and the amount of specific binding was measured (non-specific binding was evaluated by measuring binding in the presence of 1 μM unlabelled ligand). Ligand calibration curves were acquired simultaneously to obtain a measure of counts/mole ligand, which was then used to estimate receptors bound over the 1 hour incubation period.

Western Immunoblot Analysis

Proteins (35 μg) were separated by 8-10% SDS-PAGE along with Precision Plus Protein Standards (Bio-Rad Laboratories). The electrophoresed proteins were transferred to polyvinylidene fluoride membrane (Bio-Rad Laboratories) and blocked in 5% non-fat dry milk with Tris-buffered saline (10 mmol l−1Tris-HCl, 150 mmol l−1NaCl, pH 8) with 0.05% Tween 20 (TBST) at room temperature for 1 h. Immunoblot detection of GLP-1R and SUR1 were accomplished, respectively, with a rabbit anti-GLP-1R and goat anti-SUR1 affinity-purified polyclonal antibody (GLP-1R, ab13181, Abcam, Cambridge, MA, USA; SUR1, sc-5789, Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) at 1:500 and 1:50 respectively in TBST containing 5% non-fat dry milk at 4°C for 16 h. The membrane was then washed 3 times for 10 min in TBST buffer. Binding of the rabbit antiserum was detected with anti-rabbit immunoglobulin G horseradish peroxidase conjugated secondary antibody (1:20,000, 170-5046; Bio-Rad Laboratories) and binding of the goat antiserum was detected with anti-goat immunoglobulin G horseradish peroxidase conjugated secondary antibody (1:20,000, sc-2020; Santa Cruz Biotechnology, Inc.) in TBST for 1 h at room temperature and detected using SuperSignal West Pico (Thermo Fisher Scientific) exposed to Kodak x-ray film.

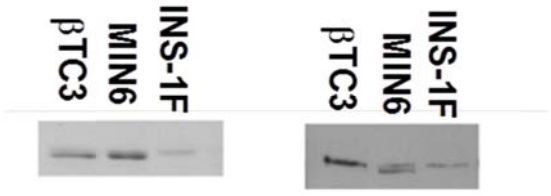

Figure S3. Western Blots for evaluation GLP-1R and SUR1 in β cell lines. Protein was extracted from cells, and thirty-five micrograms of protein was separated on a 10% (GLP1R) and 8% (SUR1) SDS-PAGE gel. After transfer to PVDF membranes, proteins were immunodetected with: Left: Abcam anti-GLP-1R (ab13181) and Right: Santa Cruz anti-SUR1 (sc-5789). The GLP-1R was identified at 53 kDa, and the SUR-1 at 175 kDa.

RNA Extraction and Quantitative Real-time PCR (qPCR)

RNA was extracted from cell lines and reverse transcribed as described previously. [2,3] Synthetic oligonucleotide primers were designed with the aid of OligoPerfect Designer software (Invitrogen Life Technologies, Carlsbad, CA, USA) and purchased from Eurofins MWG Operon (Huntsville, AL, USA; primers available upon request). The GLP-1R and SUR1 mRNA transcripts were measured by quantitative PCR using SYBR Green (Qiagen, Valencia, CA, USA) in an iQ5 Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA, USA). After the initial denaturation (95°C for 15 min), all reactions went through 40 cycles of 96°C (30 s), annealing temperature 60°C (30 s), and 72°C (10 s), at which point the

fluorescence intensity was measured. Melt curve analysis, starting at 60°C with an increase of 0.2°C every 6 s to 96°C, was performed at the end of the amplification to confirm product homogeneity. PCR efficiency and product concentration were determined with gene-specific plasmid DNA.

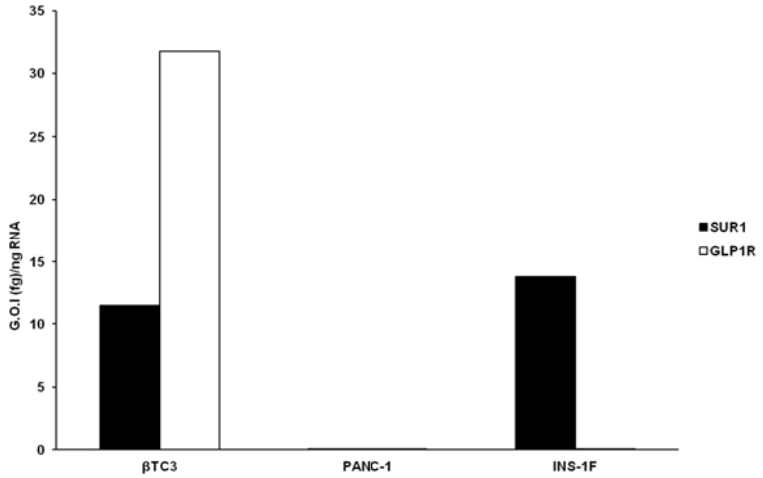

Figure S4.Cell line rt-PCR. SUR1 and GLP-1R mRNA concentrations (fmol/ng RNA) were determined by quantitative PCR in βTC3, PANC-1 and INS-1F cell lines. Level of RNA for GLP-1R in INS-1F cells was below 1 fmol/ng RNA.

Table S1. Analytical data for multivalent ligands.

[a] MS found molecular peaks (M+X)x+. [b] Peptide was eluted with a linear MeCN/0.1% CF_3CO_2H aqueous gradient (30% to 70% in 50 min) at a flow rate of 1.0 mL/min. [c] Peptide was eluted with a linear MeCN/0.1% TEAA aqueous gradient (10% to 90% in 30 min) at a flow rate of 0.3 mL/min. [d] Peptide was eluted with a linear MeCN/0.1% TEAA aqueous gradient (10% to 90% in 30 min) at a flow rate of 0.3 mL/min.

	Mass Spectrum[a]					HPLC
Compnd	Structure	Formula	Ion	Calculated	Observed	k′
9	Glp-1-Pego-$[PG]_3$-Cys(Cγ5)-Pego-Glb	$C_{268}H_{387}N_{61}O_{81}S_4Cl$	$(M+5)^{5+}$	1185.546	1185.540	9.38[b]
10	Glp-1-Pego-$[PG]_3$-Lys(Eu-DTPA)-Pego-Glg	$C_{246}H_{371}N_{61}O_{81}SClEu$	$(M+4)^{4+}$	1425.396	1425.30	5.77[c]
11	Glp-1-Pego-$[PG]_3$-Gly-Pego-Glb	$C_{228}H_{343}N_{57}O_{72}SCl$	$(M+1)^{1+}$	5099.441	5099.053	6.10[d]
12	Glp-1-Pego-(Eu-DTPA)	$C_{177}H_{271}N_{47}O_{60}SEu$	$(M+3)^{3+}$	1390.301	1390.20	5.16[c]

[1] L. Xu, J. Vagner, J. Josan, R. M. Lynch, D. L. Morse, B. Baggett, H. Han, E. A. Mash, V. J. Hruby, R. J. Gillies, *Molecular Cancer Therapeutics* **2009**, *8*, 2356–2365.

[2] L. Cole, M. Anderson, P. B. Antin, S. W. Limesand, *The Journal of Endocrinology* **2009**, *203*, 19–31.

[3] X. Chen, P. J. Rozance, W. W. Hay, S. W. Limesand, *Experimental Biology and Medicine* **2012**, *237*, 524–529.