ESM Methods

GLP-1 treatment Twenty-four week old non-diabetic βIRKO or control male mice were treated with saline or GLP-1 (Sigma, USA, 500 μmol/kg body weight) [1] via the intra-peritoneal (I.P.) route, twice a day, every 12 h, for 20 consecutive days. Blood glucose was measured before and 30 min after GLP-1 injection, and body weights were recorded every morning. IPGTT and in vivo GSIS were evaluated before and during the treatment period (GTT on day 15; GSIS on day 19). On day 21, 15 h after the last injection mice were injected with bromo-deoxy uridine (BrdU; 100 mg/kg body weight, I.P.) and five hours later the pancreases were harvested for analyses.

Cell culture Control and βIRKO beta cell lines were cultured as described [2, 3]. Throughout the experiments, no significant alteration was detected in their shape or proliferation properties. For acute stimulation experiments, cells were cultured in DMEM (Invitrogen-Thermo Fisher, Waltham, MA, USA) containing 3 mmol/l glucose +/- 10 μmol/l nifedipine (Sigma-Aldrich, St. Louis, MO, USA) 16 h prior to the experiment. Subsequently, cells were stimulated with GLP-1 (10 nmol/l) and/or insulin (human, 5 or 100 nmol/l; Sigma-Aldrich) for 15 min, and protein samples were extracted immediately. For chronic stimulation experiments, cells were cultured in DMEM containing 3 mmol/l glucose +/- exendin-4 (Sigma-Aldrich, 10 nmol/l) +/- OSI-906 (IR and IGF1R dual inhibitor, 200 nmol/l; Selleck, Houston, TX, USA) for 24 h. For protein-stability analysis the control cells were treated with 100 μg/ml cycloheximide (CHX; Sigma-Aldrich) for indicated times.

Adenovirus and lentivirus transduction After over-night culture of control cells, culture media was replaced to serum-free media and the virus was added at 500 multiplicity of infection (MOI). The virus-containing media was removed from the cells after over-night culture then regular media was added. The protein expression was analyzed by western blotting at approximately 48 h post transduction. Human wild type insulin receptor (IR) expression vectors were kindly provided by J. Whittaker, Ph.D. (Case Western Reserve University, Cleveland, OH, USA). Wild type IR open reading frames were cloned into pCDH-CMV-MCS-EF1-Puro lentiviral vector (System Biosciences, Palo Alto, CA, USA). Lentivirus particles were generated by following the manufacturer's recommendation (Open Biosystems, Huntsville, AL, USA). Cells were infected by adding the lentiviral particles to the culture with polybrene (Santa Cruz Biotechnology, Dallas, TX, USA). For generating stable cell lines, cells were treated with 4 mg/ml of puromycin 48 h after the transduction and were maintained in selection media for more than 14 days. We generated two separate stable cell lines.

REFERENCES

[1] Simonsson E, Ahren B (1998) Potentiated beta-cell response to non-glucose stimuli in insulin-resistant C57BL/6J mice. European journal of pharmacology 350: 243-250

[2] Assmann A, Ueki K, Winnay JN, Kadowaki T, Kulkarni RN (2009) Glucose effects on beta-cell growth and survival require activation of insulin receptors and insulin receptor substrate 2. Molecular and cellular biology 29: 3219-3228

[3] Liu S, Okada T, Assmann A, et al. (2009) Insulin signaling regulates mitochondrial function in pancreatic beta-cells. PLoS One 4: e7983

ESM Table 1. Antibodies for western blotting.

Antigen	Species	Provider	IDs
insulin receptor β-chain	rabbit	Cell Signaling	#3025
IGF-1 receptor	rabbit	Cell Signaling	#3124
Phospho-insulin receptor/ IGF-1 receptor	rabbit	Cell Signaling	#3024
β-actin	rabbit	Cell Signaling	#4967
cyclin A	rabbit	Santa Cruz	sc-751
cyclin D2	rabbit	Cell Signaling	#3741
cyclin E	rabbit	Santa Cruz	sc481
GLP-1 receptor	rabbit	Abcam	ab189397
EGF receptor	rabbit	Millipore	05-596
CDK2	mouse	Millipore	05-1047
Akt	rabbit	Cell Signaling	#9272
Ser473-phospho-specific Akt	rabbit	Cell Signaling	#4058
Thr308-phospho-specific Akt	rabbit	Cell Signaling	#4056
CREB	rabbit	Cell Signaling	#9197
Ser133-phospho-specific CREB	rabbit	Cell Signaling	#9198
CDK4	mouse	Cell Signaling	#2906
cyclin D1	mouse	Cell Signaling	#2926
Serine253-phospho-specific FoxO3	rabbit	Abcam	ab47285
FoxO3	rabbit	Cell Signaling	#2497
Serine9-phospho-specific GSK3β	rabbit	Cell Signaling	#9336
GSK3β	rabbit	Cell Signaling	#9315
c-Myc	mouse	Santa Cruz	sc-40
p27	rabbit	Santa Cruz	sc-1641
p57 kip2	rabbit	Abcam	ab75974
p19	mouse	Santa Cruz	sc-1066

ESM Table 2. qPCR primer sequences.

Species	Gene (Forward/ Reverse)	Sequence
mouse	β-actin forward	AGCCATGTACGTAGCCATCC
mouse	β-actin reverse	CTCTCAGCTGTGGTGGTGAA
mouse	cyclin B1 forward	AGAGGTGGAACTTGCTGAGCCT
mouse	cyclin B1 reverse	GCACATCCAGATGTTTCCATCGG
mouse	cyclin B2 forward	GCACTACCATCCTTCTCAGGTG
mouse	cyclin B2 reverse	TGTGCTGCATGACTTCCAGGAC
mouse	cyclin D1 forward	GCAGAAGGAGATTGTGCCATCC
mouse	cyclin D1 reverse	AGGAAGCGGTCCAGGTAGTTCA
mouse	cyclin D2 forward	GCAGAAGGACATCCAACCGTAC
mouse	cyclin D2 reverse	ACTCCAGCCAAGAAACGGTCCA

ESM Table 3. **Profile of random fed blood glucose**

	n	day 0	4	7	11	14	18	21	25	28	35
control + H_2O	11	6.90 ± 0.27	6.18 ± 0.26	7.44 ± 0.57	7.16 ± 0.48	6.73 ± 0.47	7.26 ± 0.37	7.51 ± 0.70	8.42 ± 1.14	8.06 ± 0.92	7.97 ± 0.86
control + VIL	12	6.77 ± 0.23	6.65 ± 0.13	6.93 ± 0.31	7.63 ± 0.53	6.99 ± 0.31	7.20 ± 0.40	9.09 ± 1.52	7.76 ± 0.56	8.04 ± 0.56	7.34 ± 0.59
βIRKO + H_2O	19	7.49 ± 0.22	7.41 ± 0.38	7.91 ± 0.39	9.39 ± 0.67	9.28 ± 0.89	8.78 ± 0.44	10.23 ± 0.66	9.48 ± 0.78	9.95 ± 0.69	7.73 ± 0.53
βIRKO + VIL	18	8.80 ± 0.42 * #	8.09 ± 0.64	8.32 ± 0.51	8.81 ± 0.69	8.59 ± 0.58	8.87 ± 0.51	9.39 ± 0.64	10.66 ± 0.65	9.70 ± 0.57	8.40 ± 0.55

mean ± SEM (mmol/l)

ESM Table 4. **Profile of body weight**

	n	day 0	4	7	11	14	18	21	25	28	35
control + H_2O	11	100	101.0 ± 0.5	103.3 ± 0.5	102.6 ± 1.3	106.6 ± 1.0	105.6 ± 1.7	108.5 ± 0.6	109.2 ± 0.8	110.7 ± 0.8	111.2 ± 0.5
control + VIL	12	100	100.8 ± 0.5	103.4 ± 0.6	104.7 ± 0.8	105.8 ± 0.8	107.9 ± 0.6	110.1 ± 0.7	110.6 ± 0.7	112.2 ± 0.8	110.7 ± 0.6
βIRKO + H_2O	19	100	101.5 ± 0.3	103.0 ± 0.5	105.1 ± 0.6	106.3 ± 0.7	107.1 ± 0.7	108.4 ± 0.6	109.1 ± 0.8	110.0 ± 0.8	108.4 ± 0.9
βIRKO + VIL	18	100	102.4 ± 0.2 #	104.5 ± 0.5	107.3 ± 0.6 *	108.2 ± 0.7	110.3 ± 0.8 *	111.9 ± 0.9 * $	113.4 ± 0.9 * $	113.8 ± 1.0	113.4 ± 1.1 $

mean ± SEM (% of day 1)

* : p<0.05 versus control+H_2O, #: p<0.05 versus control+VIL, $: p<0.05 versus βIRKO+$H_2O$

ESM Table 5. **Daily profile of random fed blood glucose**

days	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	17	18	19
control + saline	7.61 ± 0.54	7.60 ± 0.69	6.90 ± 0.44	7.22 ± 0.28	6.77 ± 0.32	6.68 ± 0.27	6.23 ± 0.23	6.32 ± 0.32	6.10 ± 0.19	6.37 ± 0.28	6.25 ± 0.22	6.62 ± 0.24	6.79 ± 0.44	6.65 ± 0.31	6.76 ± 0.18	6.49 ± 0.28	6.46 ± 0.09	6.83 ± 0.19
control + GLP-1	7.53 ± 0.32	7.54 ± 0.35	7.10 ± 0.19	6.71 ± 0.21	6.59 ± 0.17	6.42 ± 0.21	6.12 ± 0.29	6.51 ± 0.38	6.55 ± 0.44	6.55 ± 0.48	6.19 ± 0.19	6.32 ± 0.32	6.65 ± 0.35	6.34 ± 0.17	6.87 ± 0.21	6.73 ± 0.31	6.75 ± 0.31	6.99 ± 0.46
βIRKO + saline	7.81 ± 0.52	8.03 ± 0.60	8.29 ± 0.83	7.34 ± 0.68	6.85 ± 0.32	7.27 ± 0.53	6.73 ± 0.44	6.20 ± 0.21	6.47 ± 0.46	6.22 ± 0.35	6.00 ± 0.32	6.51 ± 0.37	6.73 ± 0.50	6.31 ± 0.24	6.89 ± 0.31	6.59 ± 0.20	6.70 ± 0.23	6.50 ± 0.36
βIRKO + GLP-1	6.28 ± 0.62	7.05 ± 0.59	6.90 ± 0.58	7.29 ± 0.62	7.04 ± 0.53	7.00 ± 0.42	6.82 ± 0.47	6.04 ± 0.34	6.43 ± 0.38	6.19 ± 0.44	6.18 ± 0.29	6.15 ± 0.35	6.18 ± 0.31	6.53 ± 0.33	6.21 ± 0.21	6.12 ± 0.35	6.17 ± 0.31	6.22 ± 0.25

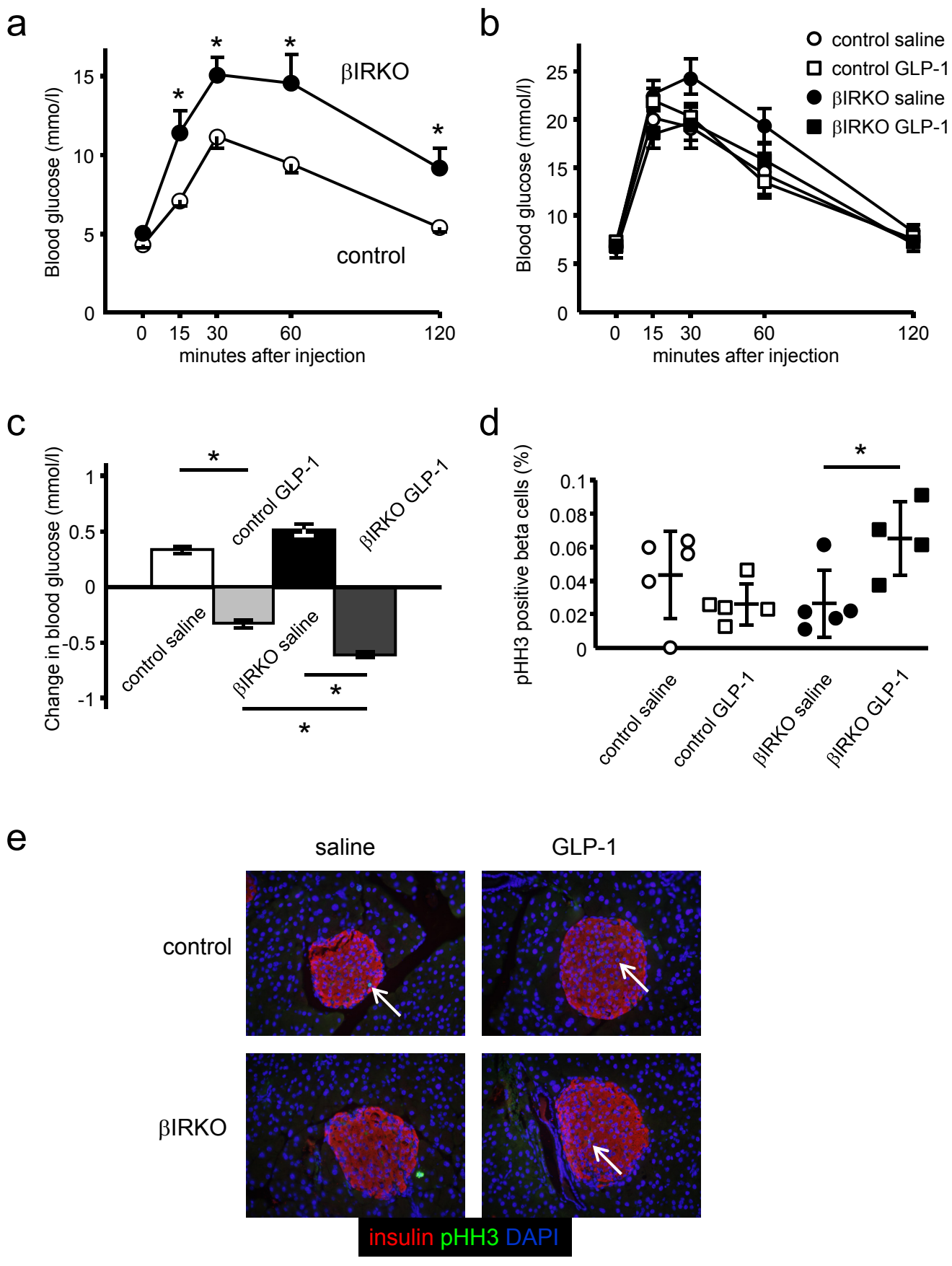
mean ± SEM (mmol/l), n=9 / each

ESM Table 6. **Daily profile of body weight**

days	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
control + saline	100	98.3 ± 0.6	96.5 ± 0.7	95.4 ± 0.8	95.5 ± 1.0	93.7 ± 1.0	93.7 ± 1.4	92.2 ± 1.3	92.3 ± 1.6	92.4 ± 1.6	91.6 ± 1.6	90.9 ± 1.7	89.8 ± 1.9	90.7 ± 2.0	88.2 ± 2.0	89.2 ± 2.4	89.9 ± 2.4	89.7 ± 2.7	86.5 ± 2.4	86.9 ± 3.2
control + GLP-1	100	97.5 ± 0.7	96.7 ± 0.8	95.3 ± 1.0	93.5 ± 1.3	93.8 ± 1.4	93.0 ± 1.6	92.7 ± 1.5	92.2 ± 1.8	92.0 ± 2.0	92.1 ± 2.0	91.1 ± 2.0	90.6 ± 2.1	92.4 ± 2.4	89.3 ± 2.8	88.6 ± 1.8	90.0 ± 2.1	89.3 ± 2.1	86.6 ± 2.1	85.9 ± 2.1
βIRKO + saline	100	98.2 ± 0.6	96.6 ± 0.8	94.7 ± 1.0	93.5 ± 0.9	93.5 ± 1.1	92.6 ± 1.1	91.1 ± 1.2	90.3 ± 1.4	90.2 ± 1.4	90.3 ± 1.5	90.1 ± 1.6	88.7 ± 1.9	88.9 ± 1.9	86.8 ± 1.8	85.9 ± 1.6	86.4 ± 1.5	86.6 ± 1.5	84.1 ± 1.4	82.7 ± 1.6
βIRKO + GLP-1	100	99.8 ± 0.5	98.3 ± 0.7	96.7 ± 0.7	95.9 ± 1.0	95.2 ± 1.1	94.4 ± 1.3	93.7 ± 1.2	94.2 ± 1.4	93.1 ± 1.1	93.5 ± 1.5	92.5 ± 1.4	92.1 ± 1.7	92.6 ± 1.6	89.9 ± 1.6	89.7 ± 1.8	90.5 ± 1.6	90.9 ± 2.0	88.3 ± 1.9	87.7 ± 2.1

mean ± SEM (% of day 1), n=9 / each

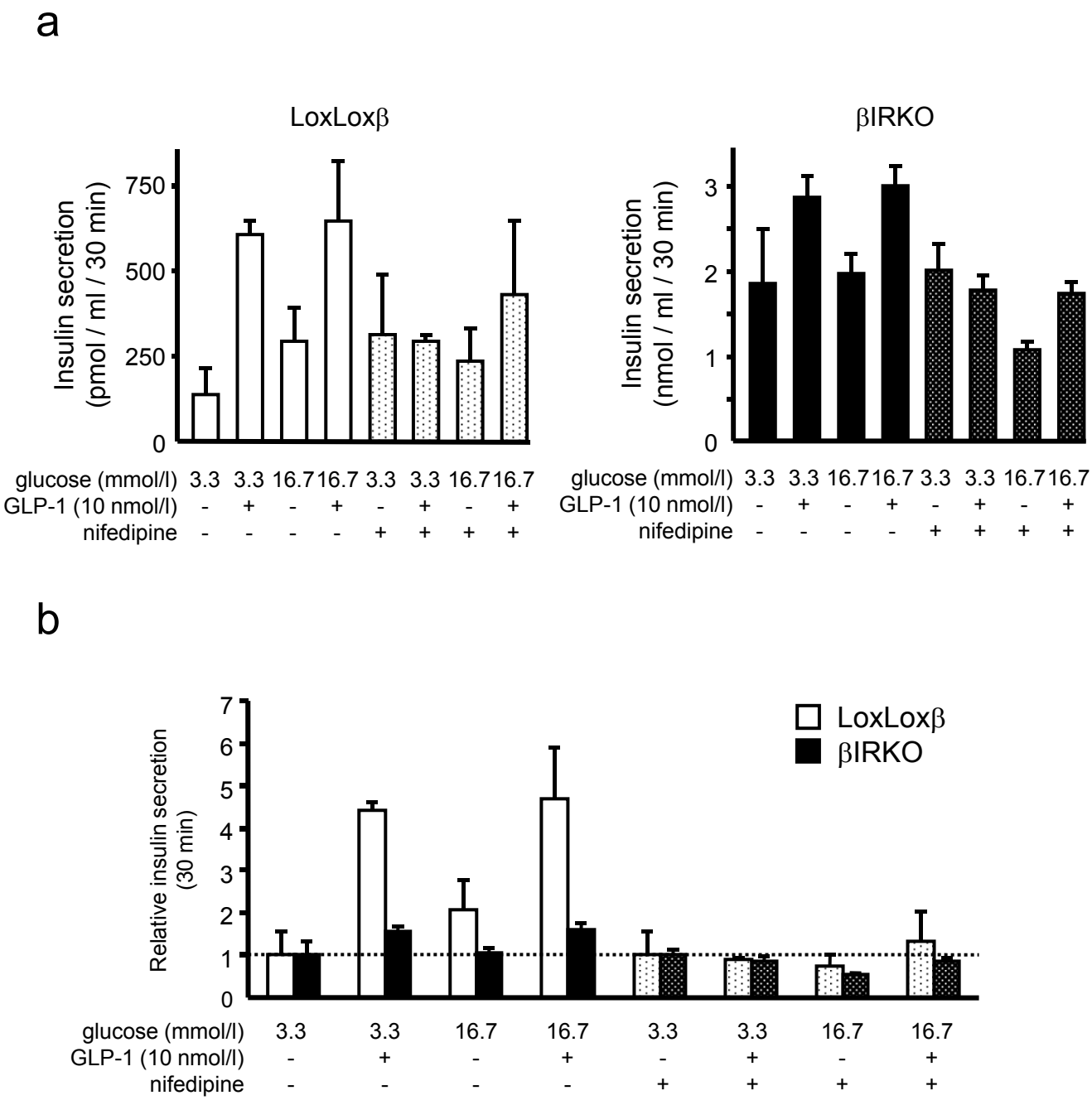
ESM Fig. 1


a
Blood glucose (mmol/l)
βIRKO
control
minutes after injection
b
control saline
control GLP-1
βIRKO saline
βIRKO GLP-1
Blood glucose (mmol/l)
minutes after injection
c
Change in blood glucose (mmol/l)
control saline
control GLP-1
βIRKO saline
βIRKO GLP-1
d
pHH3 positive beta cells (%)
control saline
control GLP-1
βIRKO saline
βIRKO GLP-1
e
saline
GLP-1
control
βIRKO
insulin pHH3 DAPI

ESM Figure 1. Effects of GLP-1 treatment on glycaemic parameters and beta cell proliferation in mice.

Intra-peritoneal glucose tolerance test before (**a**, n=18 each) and after (**b**, n=8-9 in each) GLP-1 treatment in mice. (**c**) Delta change in blood glucose levels 30 min after GLP-1 administration in mice (n=9 in each group, mean of 18 days is shown) in mice. Data are expressed as mean ± SEM. (**d**) % pHH3-positive beta cells in pancreas. (**e**) Co-immunostaining of insulin (red) and phospho-histone H3 (pHH3, green) with DAPI (blue) in pancreas sections from control and βIRKO mice after GLP-1 treatment course. A representative islet for each group at magnification 40x is presented. Arrows: pHH3-positive beta cells. Circles and squares indicate data of individual samples and horizontal bars indicate average of groups ± SEM. *, $p<0.05$; control versus βIRKO (**a**) or as indicated (**c**, **d**).

ESM Fig. 2



ESM Figure 2. Effect of nifedipine on insulin secretion from beta cell lines.

(**a**) Insulin secretion from cell lines with/without nifedipine treatment. (**b**) Relative secreted insulin normalized to the insulin secretion under 3.3 mmol/l glucose in each cell-type. The white bar represents insulin secretion from control LoxLoxβ cells, and the filled bar represents insulin secretion from βIRKO cells. n=3 each. Data are expressed as means ± S.E.M.