Supplemental Method:

Generation of a conditional GHR knockout mice.

A 10.8 kb *ghr* fragment containing exons 4 and 5 was PCR amplified with Pfu Turbo DNA polymerase (Stratagene) and cloned into pCR Blunt II-TOPO vector (Invitrogen). DNA sequence was confirmed by Sanger sequencing. A conditional gene-targeting vector was constructed from this 10.8 Kb ghr fragment by a novel recombineering approach adapted from Liu et al (1) developed in Copeland laboratory at NCI-Fredrick. Details of the recombineering protocols and other information can be found on the website at http://web.ncifcrf.gov/research/brb/recombineeringInformation.aspx. Briefly, the 8 kb fragment from the above pCR Blunt II-TOPO construct was cut out by BamHI and XbaI, then ligated to PL253 vector containing the thymidine kinase cassette. This subcloned genomic region was then modified in two targeting rounds using the neomycin (neo) cassettes from PL452 and PL451 vector, respectively. To insert the first single 5′ *loxP* site, a floxed *neo* cassette from PL452 containing homology to a region 5′ of *ghr* exon 4 was inserted. The *neo* cassette was then removed by transformation into arabinose-induced *Cre*-expressing SW106 cells, which leaves behind only a single *loxP* site along with the additional *EcoRI and BamHI* sites for Southern blot detection. Next, an *Frt-neo-Frt-loxP* cassette (from PL451) was inserted at the 3′ end of exon 4 using the same procedure. The transitions between genomic DNA and inserted sequences were confirmed by sequencing, and the conditional gene-targeting vector was tested for functionality by transformation into arabinose-induced SW105 (Flp expression; to test for neo removal) and SW106 (to test for deletion of exon 4). The conditional targeting vector was then linearized by

Not-I digestion and electroporated into B6/129 F1 hybrid *V6.5* embryonic stem (ES) cells using standard procedures. G418 (200 µg/ml)-resistant clones were analyzed by Southern blot hybridization, using external 5'probes. Correctly targeted clones were confirmed by PCR genotyping to verify the junction regions including the first LoxP site, *ghr* exon 4, two Frt sites (neo cassette) and the second LoxP site.

Two correct ES targeting clones (102 and 112) were injected into C57BL/6 blastocysts using standard procedures. After germ line transmission of the targeted (neo) allele, the conditional knockout (LL) alleles were obtained by crossing to high-efficient FLPo deleter mice to remove the Neo cassette (2). PCR condition is: 95 °C 2 min, 95 °C 20'', 57 °C 30", 72 °C 2'30", Cycles 35, 72 °C 7 min and 4 °C infinitively.

Generation of ghr β-cell specific knockout (βGHRKO) mice: Homozygous mice for the floxed *ghr* allele (after removal of the *neo*-cassette) were bred with animals carrying the RIP-Cre transgene (3-5) (gift from Dr. Pedro L.Herrera and Dr. Domenico Accili at Columbia University, New York) to produce β-cell specific *ghr* knockout mice.

Ex-vivo glucose- and arginine-stimulated insulin secretion (GSIS):
Isolation of pancreatic islets was achieved using a modified protocol published elsewhere (6). Briefly, a 1mg/ml collagenase solution (Sigma C-9263) was injected into the bile duct to perfuse the pancreas. The pancreas was then incubated with collagenase (0.8 mg/ml) at 37ºC for 20 min and then placed on ice-cold RPMI 1640 medium containing 10% fetal bovine serum. Pancreata were dispersed by pipetting and islets were picked, washed in RPMI medium Supplemented with 10% FBS, 100 U/ml Penicillin,100 U/ml Streptomycin and 5 mM glucose, and cultured overnight at

37ºC in 5% CO2 . Subsequently, 15 size-matched small and medium islets were cultured in RPMI medium and were equilibrated with Krebs Ringer Buffer (KRB; 128.8 mM NaCl, 4.8 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 2.5 mM $CaCl_2$, 5 mM $NaHCO_3$, 10 mM HEPES, and 0.1% BSA) plus 2.8 mM glucose for 30 min, prior to incubation in 500 µl KRB plus 2.8 mM glucose for 1 hr. The Supernatant was removed and stored, and islets were stimulated with 500 µl of KRB Supplemented with 5.6 mM, 11.2mM, 16.8 mM glucose, 23 mM glucose, 1µM glibenclamide (Sigma G-0639) in the presence of 23 mM glucose, or 30mM KCl in the presence of 23 mM glucose for 1 hr respectively. All steps were performed at 37°C in a tissue culture incubator. Three independent wells were used per condition. The arginine-stimulated insulin secretion was performed in the same manner as GSIS. For all experiments, total insulin was harvested by acid-ethanol extraction overnight. Insulin measurements were performed as described above.

Histology and immunohistochemistry: Tissues were fixed in 10% buffered formalin phosphate (Fisher Scientific) and embedded in paraffin. Five µm sections were used for H&E and immunohistochemistry staining. β cell mass was calculated using a modified protocol published in JCI (7, 8). Briefly, 3-5 pancreatic sections per mouse were stained with anti-insulin antibody. Relative β cell area was calculated using NIH Image J software (http://rsb.info.nih.gov/nih-image/) and β cell mass was calculated as β cell area multiplied by pancreatic weight. We used 6 mice per group. Primary antibodies: rabbit anti-human insulin (Cell Signaling C27C9) or goat anti-human insulin A (Santa Cruz sc-7839), goat anti-mouse GHr (Sigma S8919), rabbit anti-phospho-Stat5 (Cell

Signaling 9359), mouse monoclonal anti-Cre (VWR 69050), mouse monoclonal anti-POMC (Abcam ab73092), rabbit anti-NPY (Phoenix Biotech H-049-03) and goat anti-Glut2 antibody (Santa Cruz sc-7580), rabbit anti-cyclin D2 antibody (Santa Cruz sc-593), rabbit anti-Ki67 antibody, (Vector Lab, VP-RM 04). Secondary antibodies: donkey anti-rabbit Alexa 488 (Invitrogen A-21206), and chicken anti-goat Alexa 594 (Invitrogen A-21468). Samples were viewed under bright-field illumination or optical filters (immunofluorescence) using an Olympus AX70 microscope and digital DP71 camera with the MicroSuite™ software.

Gene expression studies: RNA was isolated from tissues or isolated islets using TRIzol reagent according to the manufacturer's instructions (Invitrogen). RNA integrity was verified using Bioanalyzer (Agilent Technologies). One ug of RNA was reverse-transcribed to cDNA using oligo(dT) primers with RT-PCR kit according to the manufacturer's instructions (Invitrogen). Real-time PCR was performed with the QuantiTect™ SYBR® green PCR kit (Qiagen) according to the manufacturer's instructions in ABI PRISM 7900HT sequence detection systems (Applied Biosystems). Each transcript in each sample was assayed 3 times and the fold-change ratios between experimental and control samples for each gene used in the analysis were calculated using β-actin or glucose-3-phosphate dehydrogenase (G3DPH) levels as a reference. Sequences of primers used for real time PCR are available in Supplement table 1.

REFERENCES:

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7.   Maier B, Ogihara T, Trace AP, *et al.* The unique hypusine modification of eIF5A promotes islet beta cell inflammation and dysfunction in mice. The Journal of clinical investigation 2010;120(6):2156-70.

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Supplemental table 1: Real-time PCR primer sequence:

mCNND2-F	CGCTACCGACTTCAAGTTTGC
mCNND2R	CAATCCACATCAGTGTGGGTG
mGCK-F	AAAGATGTTGCCCACCTACG
mGCK-R	TCTGGTGTTTCGTCTTCACG
mIRS2-F	GAAGCGGCTAAGTCTCATGGA
mIRS2-R	TGACGGTGGTGGTAGAGGAAA
mKir6.2-F	GACCTCAAATGGCCACACACT
mKir6.2-R	CAGCCAGCTGCACAGGAA
mSUR1-F	TGCTGCAAAGGACGTTCCT
mSUR1-R	CCTCTCAGGTTAATCCCAGTTTCA
mGHR-F	GGAGACATCCAAGTGAGTTGG
mGHR-R	ATCCATTCTCAATGAGTACAC
Cre-F	GCGTTCGAACGCACTGATTTC
Cre-R	TACACCAGAGACGGAAATCCA
mNPY-F	CTCCGCTCTGCGACACTAC
mNPY-R	AATCAGTGTCTCAGGGCT
mAGRP-F	GCGGAGGTGCTAGATCCA
mAGRP-R	AGGACTCGTGCAGCCTTA
mPOMC-F	TAAGAGCAGTGACTAAGAGAGGC
mPOMC-R	CACACATCTATGGAGGTCTGAAG
mG3PDH-F	TGAAGGTCGGTGTCAACGGATTTGGC
mG3PDH-R	CATGTAGGCCATGAGGTCCACCAC
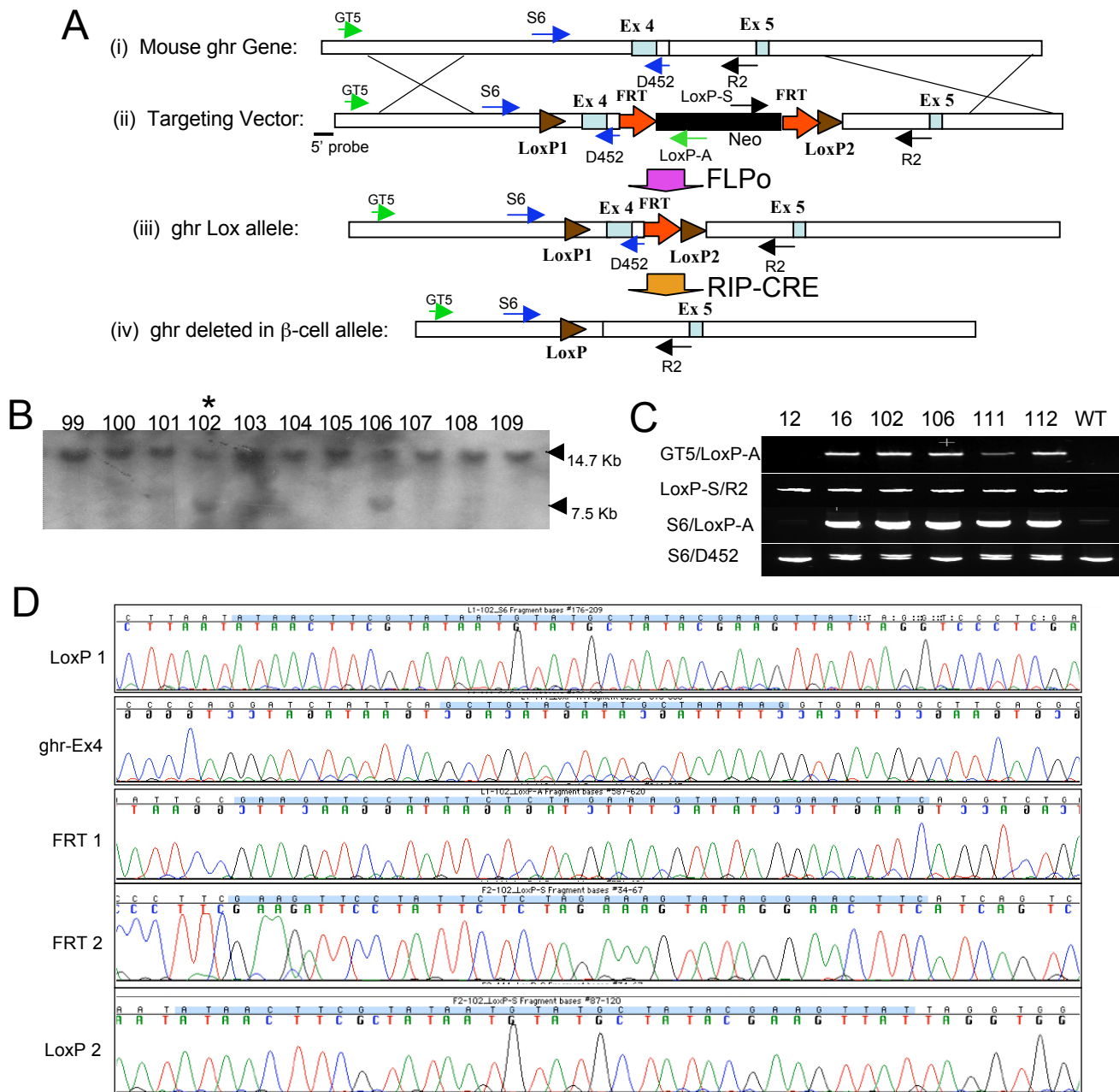
Supplemental Figure 1: Generation of a conditional GHR knockout mice.
(A) Schematic diagram of construction of β-cell specific GHR knockout mice. (i) The mouse *ghr* locus surrounding exon 4 (Ex4) and exon 5 (Ex5) is shown. (ii) In the targeting vector, exon 4 was flanked by LoxP sites along with FRT site-flanked Neo cassette. (iii) After crossing with FLPo deleter mice, the Neo cassette was removed. (iv) Exon 4 was excised in β cells by crossing mice homozygous for the *ghr* floxed allele with mice expressing Cre under the rat insulin II promoter (RIP-CRE). Knocking out Exon 4 results in a frame shift that generates a premature stop codon in exon 6. Primers GT5, S6, D452, LoxP-A and R2 for PCR genotyping are indicated. Red arrow: FRT sites; Brown triangle: LoxP sites. (B) Southern blot screen of targeted ES clones was performed using a probe external to the 5′ end of the homology region (thick black line in A) and ES clone genomic DNA was digested with Xba I. The wild-type and targeted alleles are indicated as 14.7 Kb and 7.5 Kb fragments, respectively. (C) Positive ES clones were confirmed by PCR analysis. Wild type ES cells (WT) were used as control. GT5/LoxP-A, LoxP-S/R2 and S6/LoxP-A are specific primers for the amplification of the Neo cassette junction region. S6/D452 primers distinguish between WT, Neo and the Lox alleles. (D) Sequence confirmation of floxed allele at the junctions of LoxP sites, FRT sites and Exon 4 region.
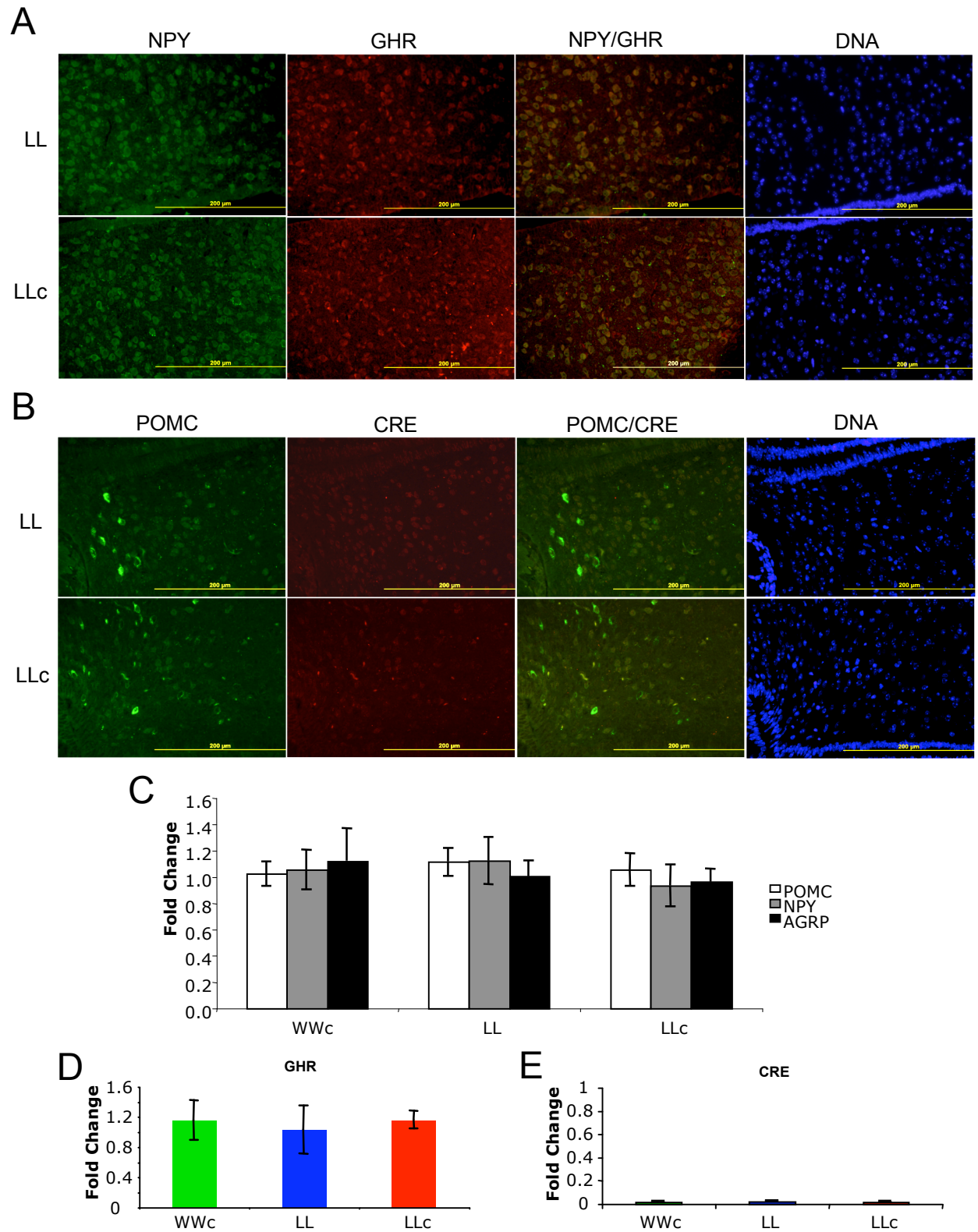
Supplemental Figure 2: βGHRKO mice display normal protein levels of GHR in the hypothalamus.
(A) Immunofluorecence analysis of neuropeptide-Y (NPY) in green and the GHR in red in hypothalami sections from control and βGHRKO mice. Shown are representative images from 3 mice/group. (B) Immunofluorecence analysis of POMC (green) and the CRE (red) in hypothalami sections from control and βGHRKO mice. Shown are representative images from 3 mice/group. (C) Gene expression of POMC, NPY and AGRP genes in hypothalami of control and βGHRKO mice was analyzed by real-time PCR. Gene expression of ghr (D) and the cre transgene (E) in hypothalami analyzed by real time PCR. Scale bars: 200μm (A,B).
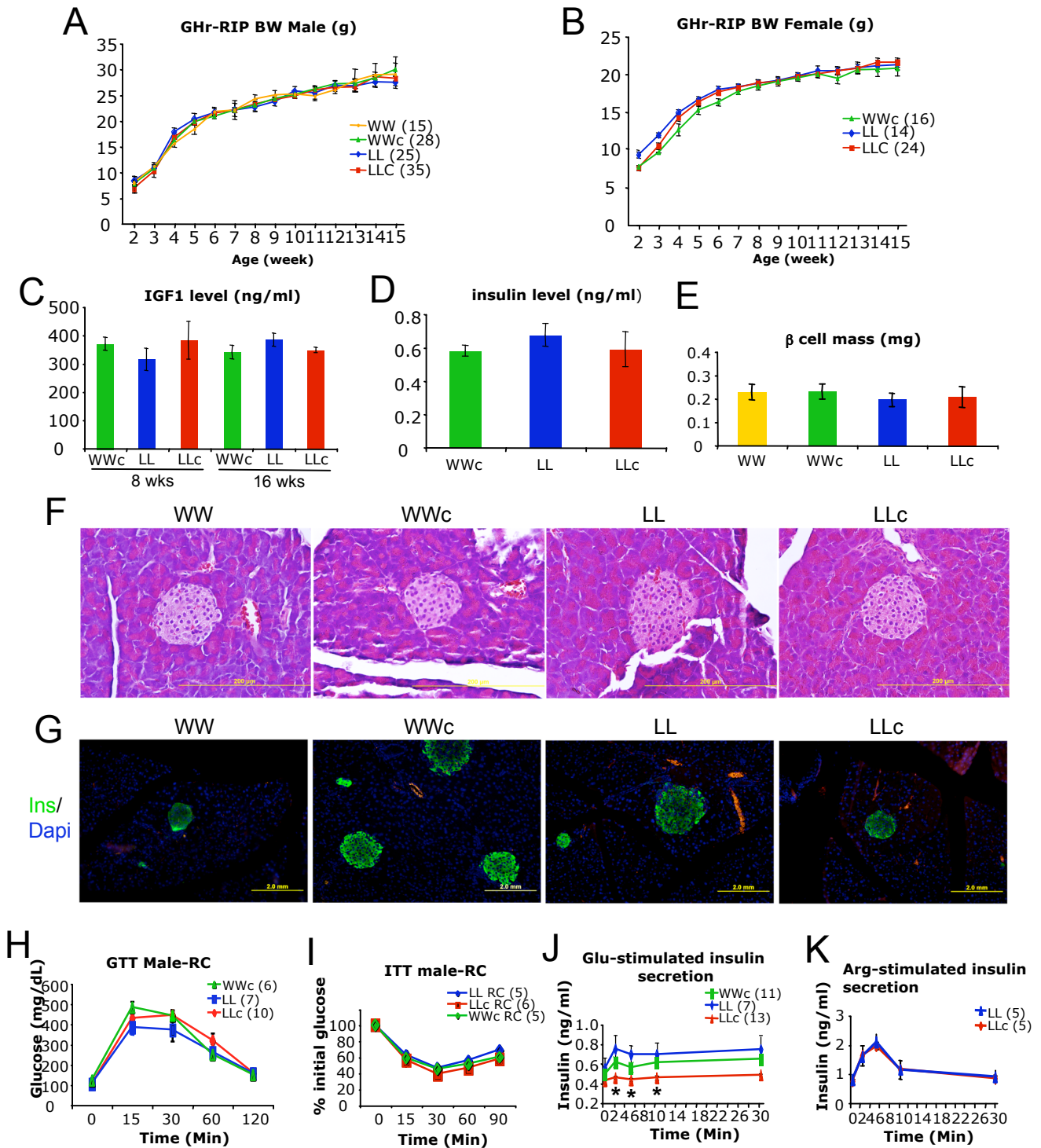
Supplemental Figure 3: Characterization of βGHRKO mice fed normal chow.
Body weight of male (A) and female (B) control and βGHRKO mice was followed weekly from 2-15 weeks of age. Serum IGF-1 (C) and insulin (D) levels were determined by RIA 6-8 mice per group. β-cell mass was quantified (E) from insulin stained pancreas sections (F). (G) Representative sections from control and βGHRKO mice at 16 weeks of age stained with anti-insulin antibody, in green. Intraperitoneal glucose tolerance test (H) and Intraperitoneal insulin tolerance test (I) was performed in 16 weeks old control and βGHRKO mice. (J) First-phase glucose stimulated insulin secretion was performed *in vivo* in 16 week old male control and βGHRKO mice. (K) Ariginine-stimulated insulin secretion was measured *in vivo* in 16 week old male control and βGHRKO mice. Scale bars: 200μm (F)., 2.0mm (G).

Insulin/DNA content
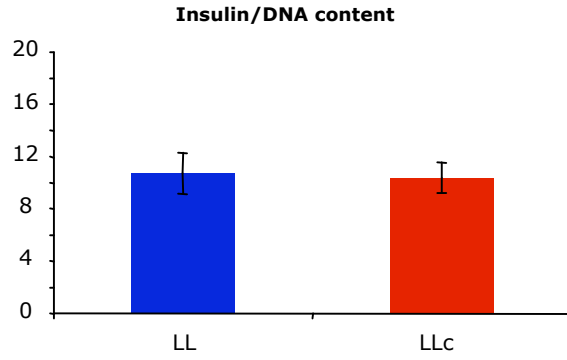
Supplemental Figure S4: **Insulin content in islet extracts in HFD-fed βGHRKO mice.**
Insulin content in islet extracts from control and βGHRKO mice was measured by RIA and corrected by DNA content. Islets were incubated in 23 mM glucose for 1 h.
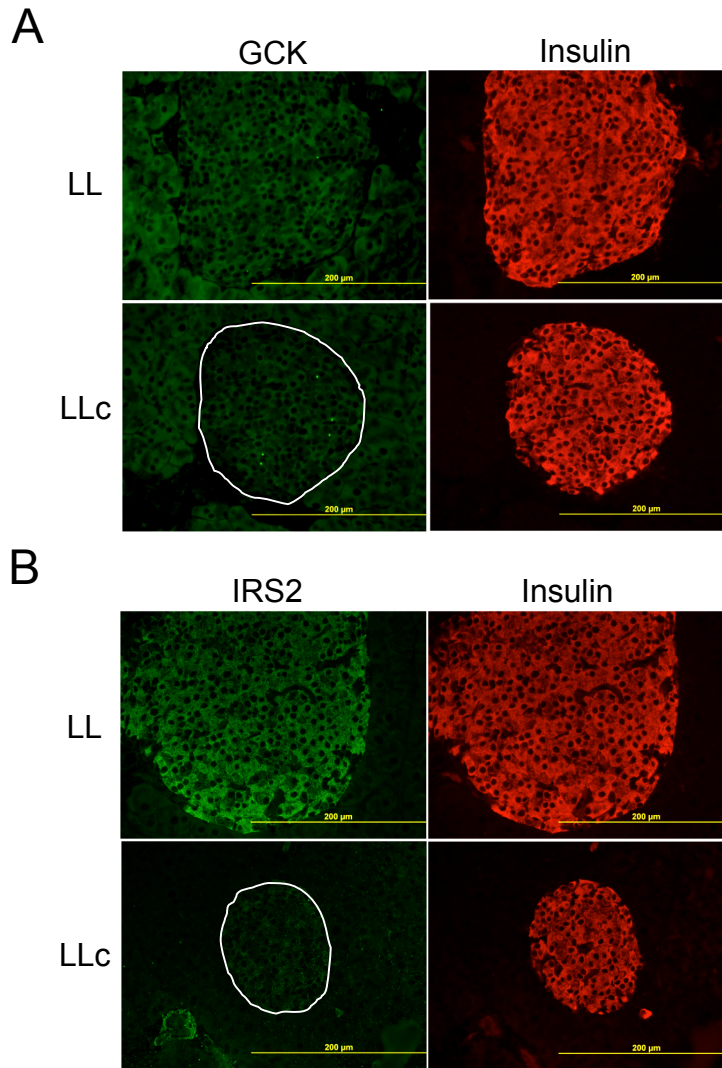
Supplemental Figure S5: **Reduced expression level of GCK and IRS2 in HFD-fed βGHRKO mice.**
Immunofluorecence analysis of GCK (A) and IRS2 (B) protein levels in pancreatic sections of obese control and βGHRKO mice. Scale bars: 200μm (A,B).