ESM Methods:

Detailed description of generation of the mutGCK mouse:

A 13.3 kb DNA fragment containing part of the *Gck* coding sequence was retrieved from C57BL/6J mouse bacterial artificial chromosome clone RP-23-373-F12 via bacterial recombination [1] into plasmid PL253 that contains an Mc1-driven thymidine kinase cassette for negative selection (ESM Fig. 1a). A cassette of two loxP sites was inserted before the native exon 10, using the PL452 plasmid. Cre activation (by arabinose supplementation), left only one loxP site upstream the Exon 10 (ESM Fig. 1a).

We next introduced the three nucleotides cgg into amino acid 454 of exon 10 of the *Gck* gene in the plasmid using the Quickchange-site directed mutagenesis kit (Stratagene, California, USA, cat: 200523), with the following primers: 5'-ACTGGTCTCTGCGGCGGTGGCCTGCAAG-3' and 5'-CTTGCAGGCCACCGCCG-CAGAGACCAGT-3'. This mutation, ins454A, causes severe GCK-CHI in humans [2].

A cassette including one LoxP site followed by neo selection cassette, *Gck* exon 10 containing the ins454A mutation and IRES2-AcGFP (Clontech, Shiga, Japan, cat: 632435) was generated using the PL452 plasmid and inserted by bacterial recombination immediately down-stream from the native intron 10 (ESM Fig. 1a). The linearized targeting vector (ESM Fig. 1b) was electroporated into B6 embryonic stem cells (ESCs) (Chemicon; Massachusetts, USA), and clones that survived G418 and ganciclovir selection were screened for homologous recombination by PCR (ESM Fig. 2a,b) and Southern blot analysis (ESM Fig. 2b). Targeted clones were injected into C57BL/6J-derived blastocysts that were then transferred to pseudo pregnant females. Male offspring were mated to C57BL/6J females, and ESC-derived offspring were identified by PCR-based genotyping (ESM Fig. 3). Mice homozygous for the targeted insertion of the ins454A severe mutation and the two loxP sites in the *Gck* gene locus were then crossed either with mice expressing Cre recombinase driven by the rat insulin promoter (RIP-Cre) or with a mouse expressing estrogen-dependent Cre driven by the mouse insulin promoter (MIP-CreER), as described in the main text, to achieve removal of the wild type exon 10 and expression of mutant exon 10 in the natively regulated glucokinase locus. Genotyping of all offspring was performed by PCR for the AcGFP sequence, using the following primers: forward: 5-ATCCTGATCGAGCTGAATGG-3; reverse: 5-TGGGTGGACAGGTAGTGGTT-3, which yield a 573bp product in the transgene allele. All PCR reactions were performed for 35

cycles under the following conditions: 94°C 2'; 35x (94°C 30'', 60°C 30'', 72°C 30''); 72°C 5'.

[1] Copeland NG, Jenkins NA, Court DL (2001) Recombineering: a powerful new tool for mouse functional genomics. Nat Rev Genet 2(10): 769-779. 10.1038/35093556

[2] Sayed S, Langdon DR, Odili S, et al. (2009) Extremes of clinical and enzymatic phenotypes in children with hyperinsulinism caused by glucokinase activating mutations. Diabetes 58(6): 1419-1427. 10.2337/db08-1792

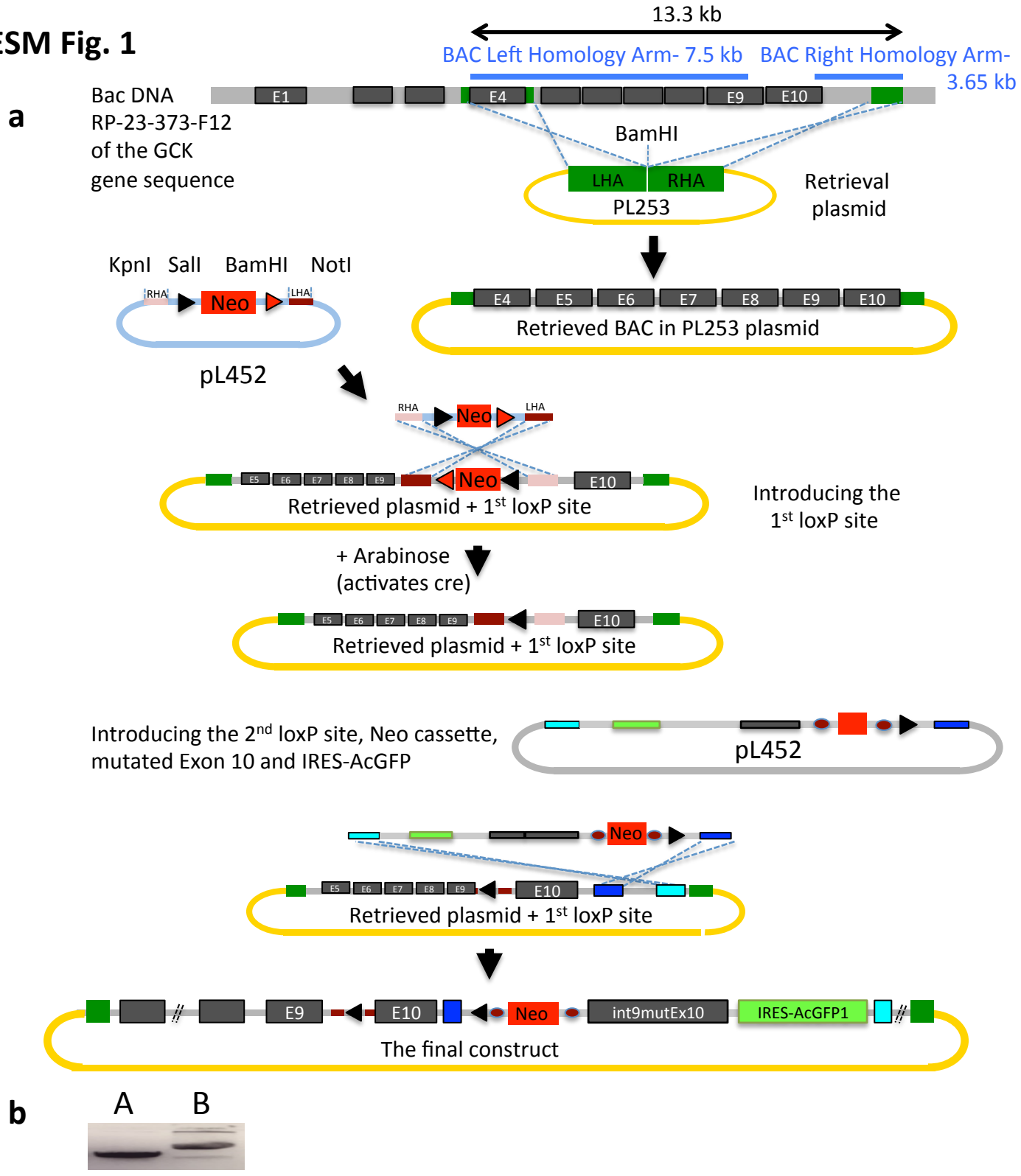
Table 1 Antibodies used for immunostaining

Antibody	Dilution	Source
Guinea pig anti insulin	1:200	Abcam ab7842, Cambridge, UK; Dako A0564, Santa Clara, CA 95051 USA
Rabbit anti Ki67	1:200	Thermo 9106, Thermo Fisher Scientific, Massachusetts, USA
Rabbit anti P53	1:400	Bethyl Texas, USA
Secondary antibodies	1:200/500	Jackson Immunoresearch, Pennsylvania, USA
DAPI to identify nuclei	1:100	Sigma MBD0015

Table 2 Primers used for qPCR analyses

Gene	Forward primer 5′ to 3′	Reverse primer 5′ to 3′
β-actin	CACAGCTTCTTTGCAGCTCCT	GTCATCCATGGCGAACTGG
Chop	CACCACACCTGAAAGCAGAA	GGTGAAAGGCAGGGACTCA
Atf3	CAGACCCCTGGAGATGTCAGT	TTCTTGTTTCGACACTTGGCA
Total Xbp1	AAGAACACGCTTGGGAATGG	ACTCCCCTTGGCCTCCAC
Spliced Xbp1	GAGTCCGCAGCAGGTG	GTGTCAGAGTCCATGGGA

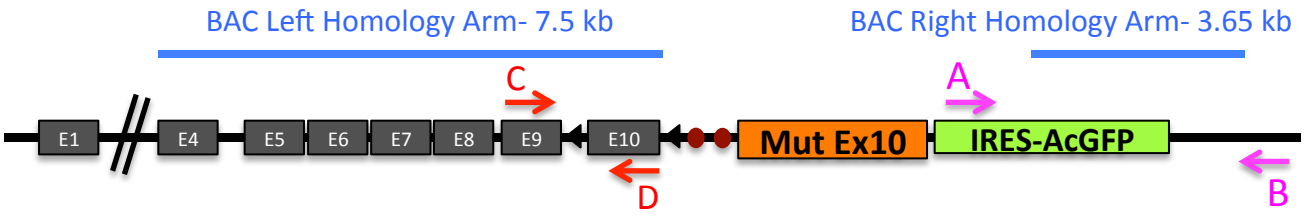
ESM Fig. 1



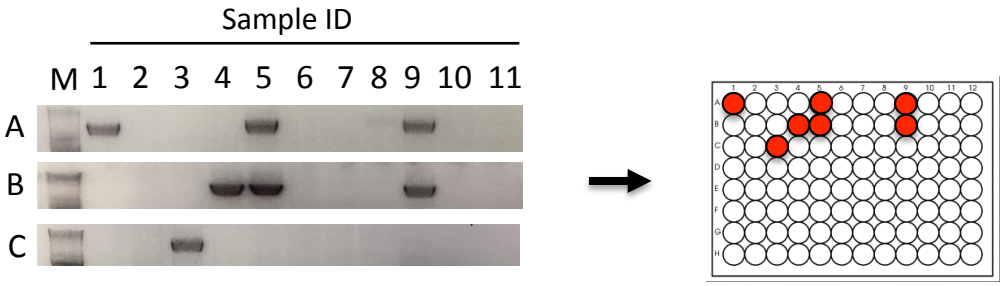
(**a**) Schematic representation of Tg INS454-GCK mouse generation by serial recombination steps: A 13.3 kb DNA fragment containing part of the GCK coding sequences was retrieved from C57BL/6J mouse bacterial artificial chromosome clone RP-23-373-F12 via bacterial recombination into plasmid PL253. The upstream loxP site was introduced using the PL452 plasmid. A cassette including mutated GCK exon 10 followed by IRES-AcGFP was inserted immediately downstream to the native exon 10, which was flanked by a second LoxP site (Black triangles). (**b**) The linearized final GCK mutant targeting vector (A) and un-linearized vector (B) before the electroporation into B6 embryonic stem cells (ESCs).

ESM Fig 2

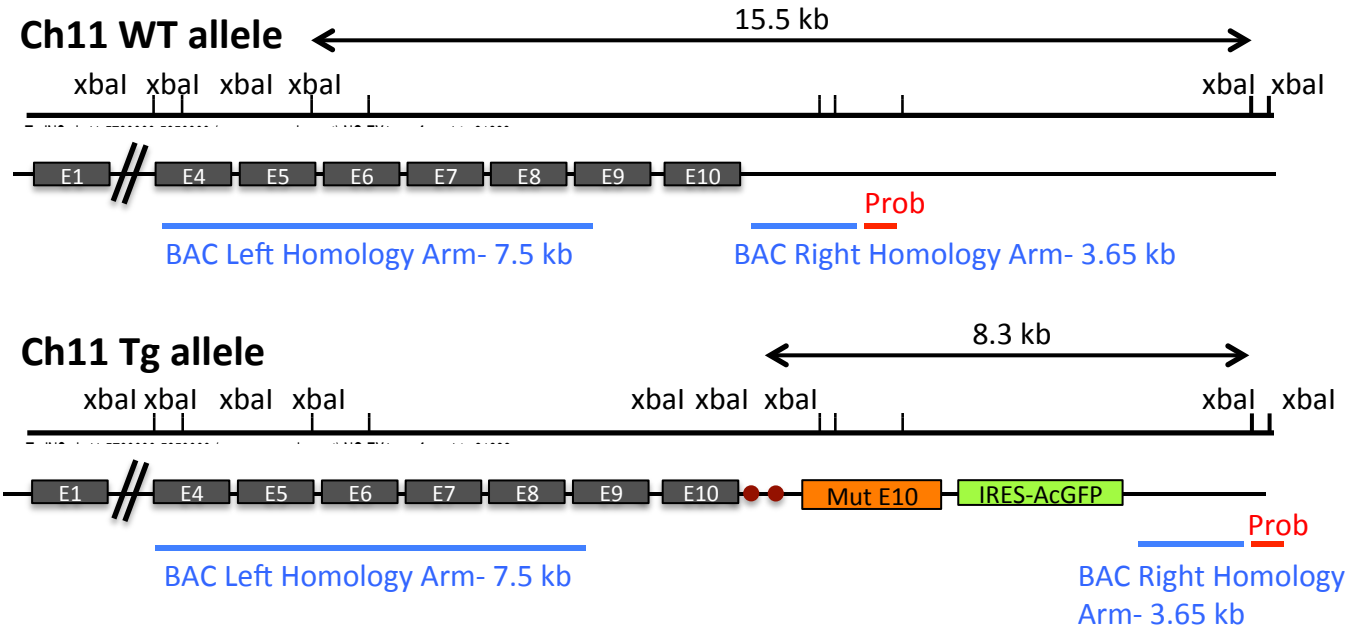
a. ES screening by PCR - Ch11 Tg allele - Primers design

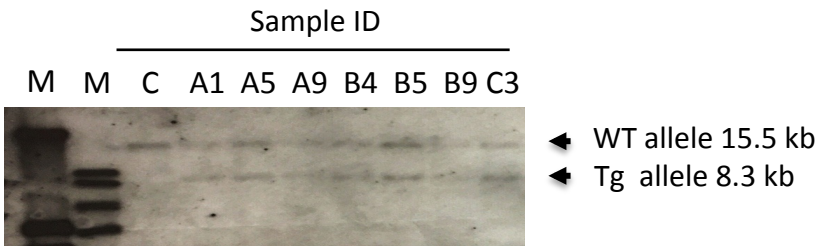


b. positive PCR results using primers pair A+B



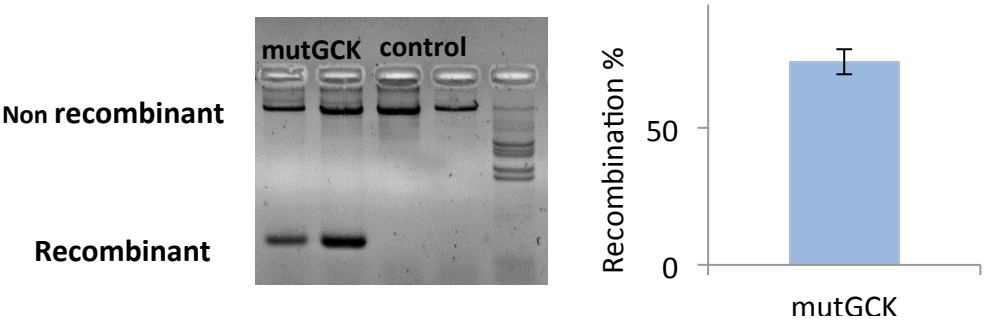
c.





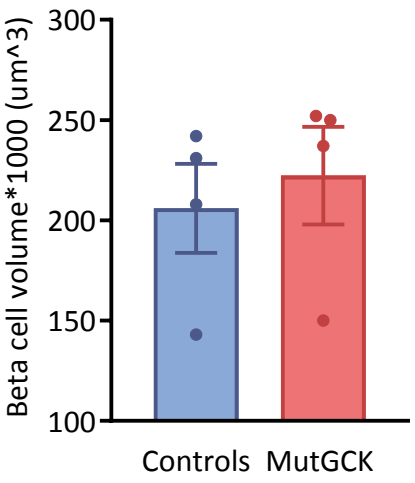
(**a**) Schematic map of the final mutated GCK construct. BAC homology sequence are marked in blue. ES clones that survived G418 and ganciclovir selection were screened for homologous recombination by PCR (**b**). The PCR primers are marked in red and pink arrows in the upper scheme. (**c**) Positive PCR-ES colonies were then screened by Southern Blot analysis.

ESM Fig. 3



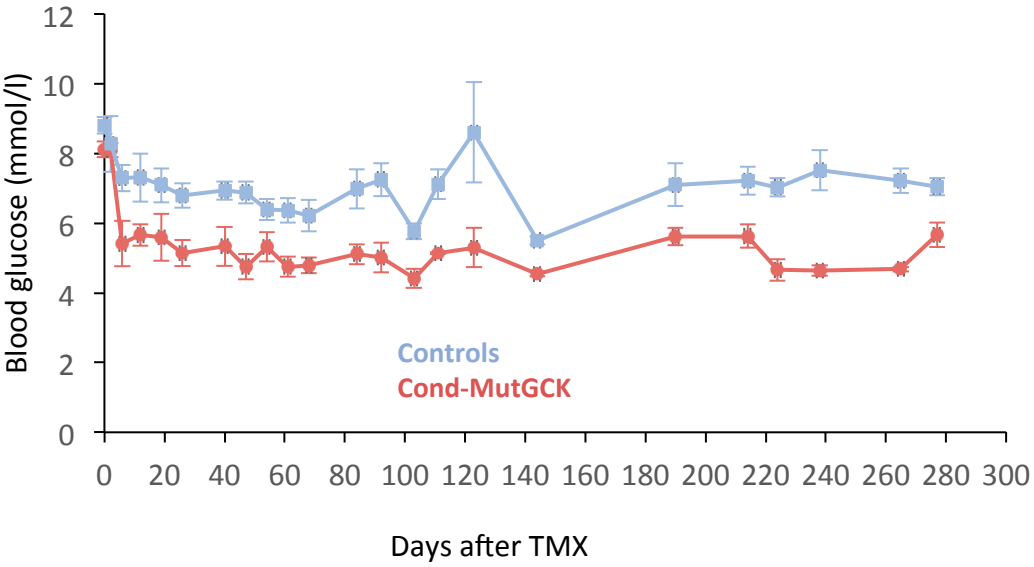
Left: Gel electrophoresis of amplificons following PCR amblification using primers indicated in Figure 1A, confirming recombination after expression of Cre recombinase. **Right:** Quantification of amplicons demonstrating approximately 75% recombination efficiency.

ESM Fig. 4



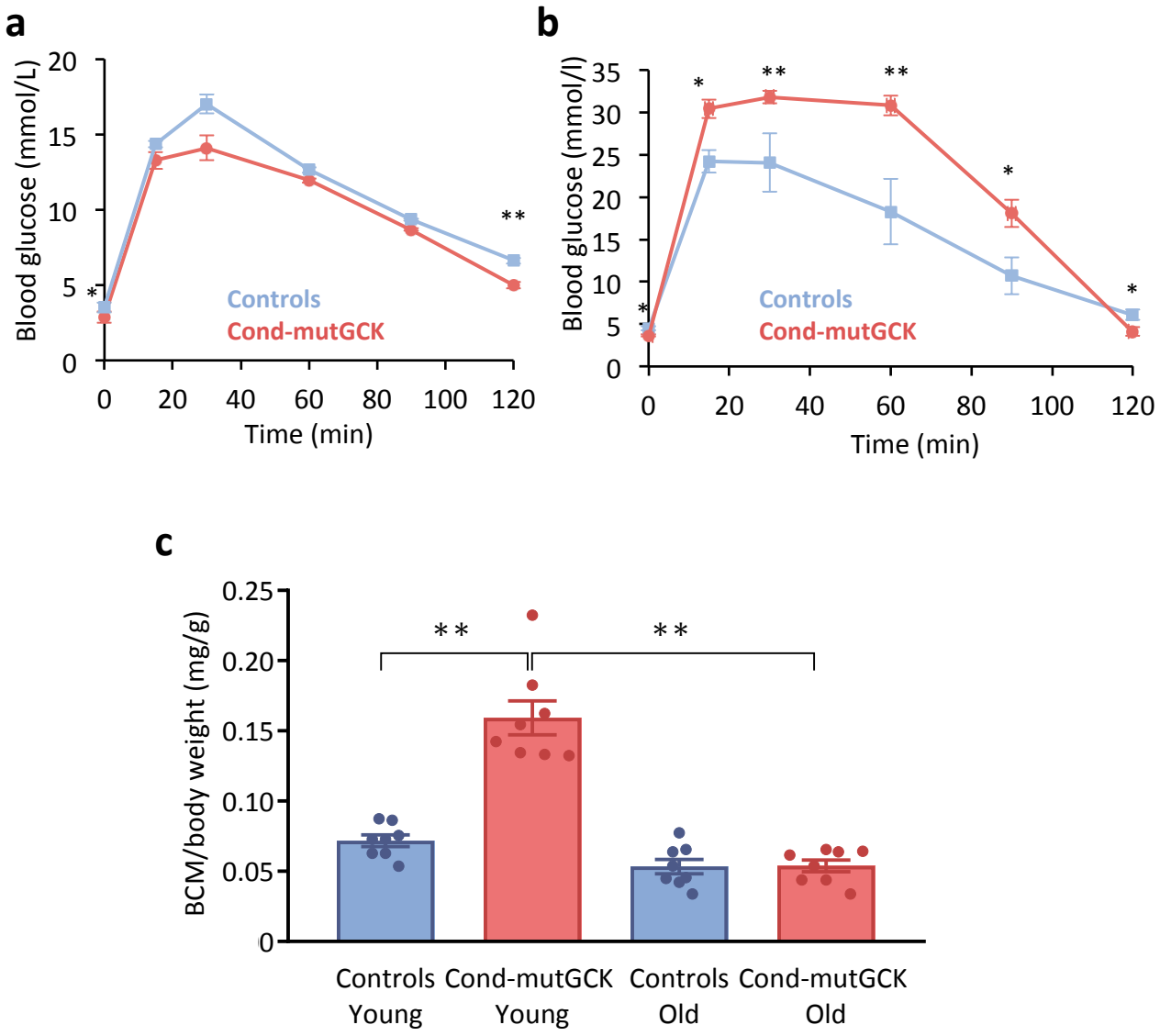
Estimated beta cell volume in control and 1.5 month old beta-mutGCK mice. These data suggest that the increased beta cell mass seen in these mice was not due to beta cell hypertrophy.

ESM Fig 5



Long-term, persistent hypoglycemia following cre-recombination in cond-beta-mutGCK mice. Tamoxifen was injected at day 0 and day 2. n=8 cond-beta-mutGCK and 8 control mice. (Here and in subsequent supplemental figures cond-beta-mutGCK mice are designated as cond-mutGCK).

ESM Fig. 6



(**a**) IP glucose injection demonstrating normal glucose tolerance in 1.5 month old cond-beta-mutGCK mice (N=8 cond-beta-mutGCK and 8 control mice). (**b**) IP glucose injection demonstrating abnormal glucose tolerance in 9 month old cond-beta-mutGCK mice (N=8 cond-beta-mutGCK and 8 control mice). (**c**) Beta-cell mass in 1.5 and 9 month old cond-beta-mutGCK and control mice (N=8 cond-beta-mutGCK and 8 control mice).