SUPPLEMENTARY DATA

Materials and methods

Isolation of pancreatic islets (1)

Mice were anesthetized with ketamine/Xylazine (25mg/ml each, 2ul/g body weight). Internal organs were exposed after a typical V-incision. Bile ducts were identified and the entry point of the ducts into the gut was clamped. 3~5ml of cold 1.4mg/ml collagenase P (Roche) in 1xHBSS (Invitrogen) supplemented with 0.35g $NaHCO_3$/L and 1% BSA (Sigma) was infused into the duct leading into the pancreas via a 30 gauge needle. Inflated pancreas was dissected and kept on ice before digested at 37 °C for 8min. Cold RPMI 1640 (Invitrogen) supplemented with 1% BSA (Sigma) was used to stop the digestion and wash the pancreas (3 times). Islets were hand-picked under a dissection microscope and kept in supplemented RPMI 1640 in the incubator before use.

Insulin mRNA quantification from isolated pancreatic islets

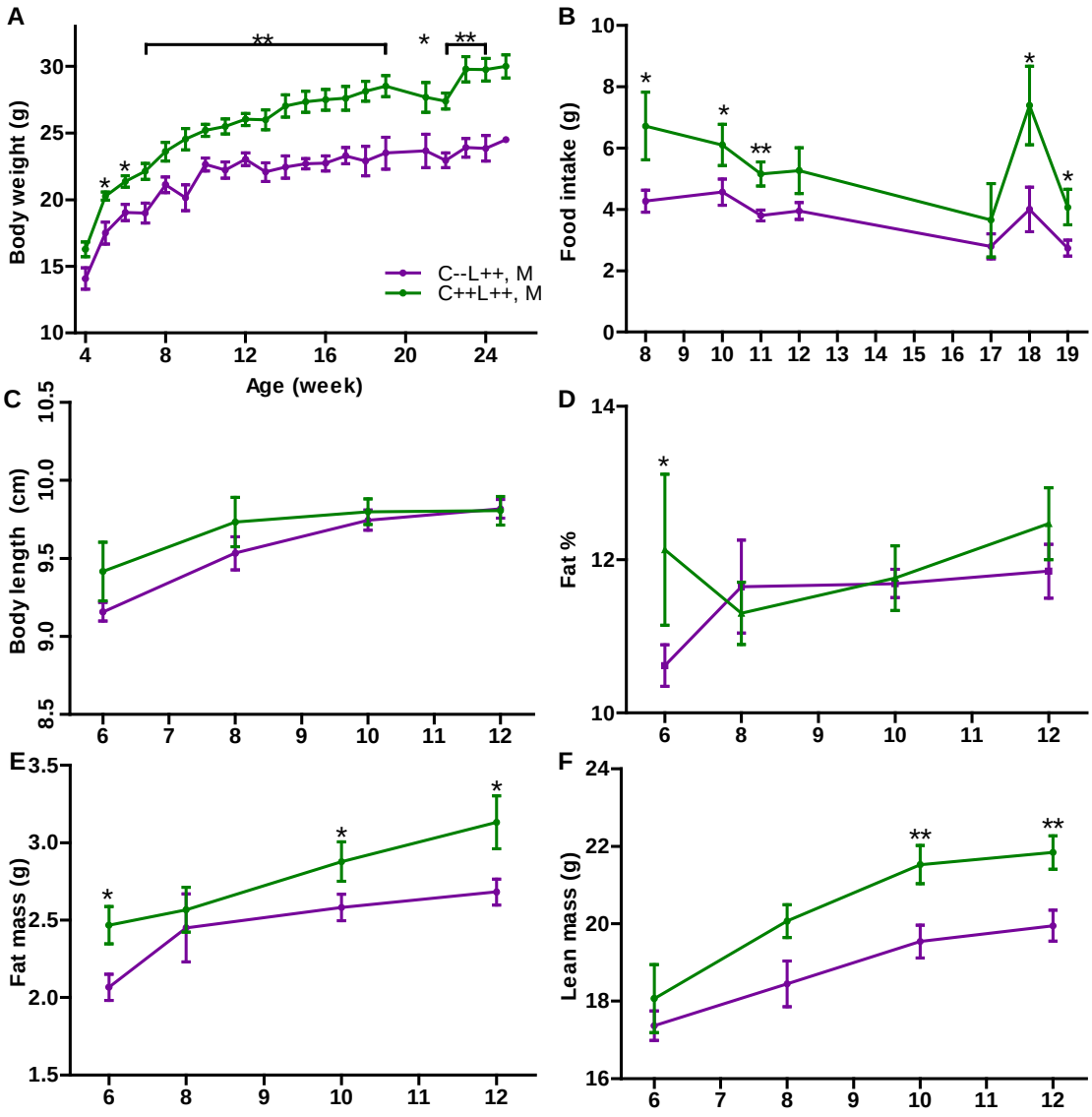
50 islets from each mouse were picked after overnight culturing. RNA extraction, purification, reverse transcription, cDNA purification and real-time PCR were carried out as described for the liver. Mouse Insulin Taqman Gene Expression Assay Mm00731595_gH was used. Results were normalized to HPRT mRNA.

Glucose-stimulated insulin secretion from isolated pancreatic islets (2)

50 islets per mouse were picked after overnight culturing and transferred into a 1.5ml tube. After incubated in 1ml of 3mM glucose in SAB buffer (114mM NaCl, 4.7mM KCl, 1.2mM KH_2PO_4, 1.16mM $MgSO_4$, 2.5mM $CaCl_2$, 25mM $NaHCO_3$, 20mM HEPES, 1% fatty acid-free BSA, pH 7.4) for 1hr at 37°C (time 0), islets were incubated in 0.5ml of 12mM glucose in SAB for another 2hr. 20ul samples collected at 30, 60, 90 and 120min. Insulin was quantified using Mouse Insulin Ultrasensitive EIA (ALPCO).

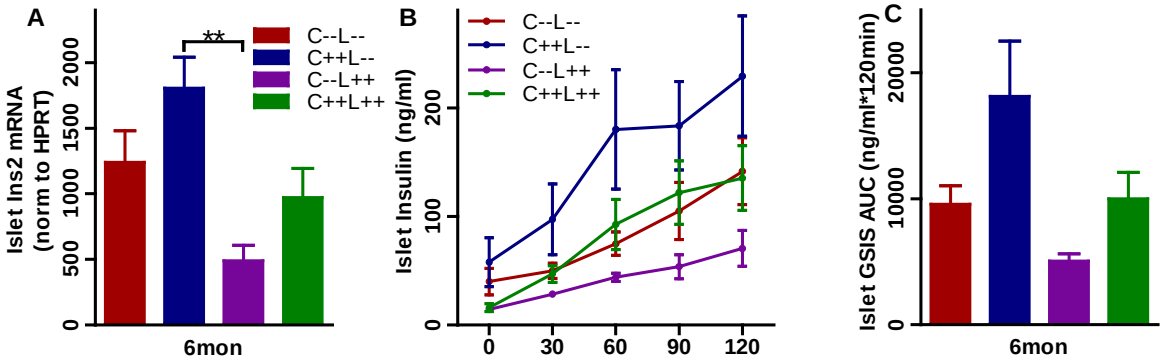
SUPPLEMENTARY DATA

Supplementary Figure 1. Growth-curve comparison between C--L++ and C++L++ males. Body weight, food intake, body length, fat percentage (Fat%), fat mass and lean mass were measured and plotted at indicated ages. A) Body weight. C--L++ mice had significantly reduced body weight compared to C++L++ mice (*, **: $p<0.05$ or 0.01). B) Food intake. C--L++ mice consumed less food compared to C++L++ mice (*, **: $p<0.05$ or 0.01). C) Body length. C--L++ and C++L++ had similar body length ($p>0.05$). D) Fat%. Percentage of fat relative to total body weight was lower in C--L++ vs. C++L++ mice at 6 week of age (*: $p<0.05$). E) Fat mass. C--L++ mice showed reduced fat mass compared to C++L++ mice (*: $p<0.05$). F) Lean mass. C--L++ mice had reduced lean mass compared to C++L++ (**: $p<0.01$).



SUPPLEMENTARY DATA

Supplementary Figure 2. Quantitation of insulin mRNA and GSIS in isolated pancreatic islets. Pancreatic islets were isolated from 5 to 7 mice for each genotype. 50 islets were used for total RNA extraction or glucose-stimulated insulin secretion. A) Insulin mRNA from islets under non-stimulation condition was quantitated relative to HPRT mRNA. C--L-- and C--L++ showed reduced insulin mRNA compared to C++L-- and C++L++ respectively, but neither difference reached statistical significance. C++L-- mice showed significantly increased insulin mRNA compared to C--L++ mice (**: $p<0.01$). B) GSIS time course of isolated islets from all four genotypes. No significant difference was observed between any genotypes. C) AUC of GSIS. C--L-- and C--L++ showed reduced insulin secretion compared to C++L-- and C++L++ respectively, but neither difference reached statistical significance.



References

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