Supplemental material

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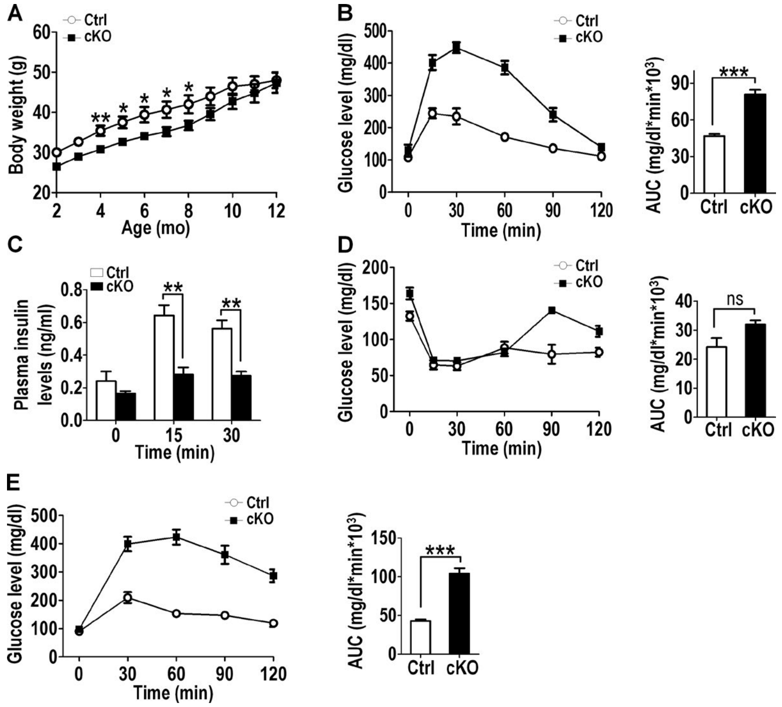

Figure S1. **Sex and age do not affect the phenotype.** (A) Body weight was measured at indicated age (Ctrl and cKO, *n* = 12, male). (B) IPGTT was performed on 8-wk-old female mice (control [Ctrl] and cKO, *n* = 6). Area under the curve (AUC) shown at right. (C) Plasma insulin levels in female mice during IPGTT (Ctrl and cKO, *n* = 5). (D) IPITT was performed on 8-wk-old female mice (Ctrl and cKO, *n* = 6). Area under the curve shown at right. (E) IPGTT was performed on 10-mo-old male mice (Ctrl and cKO, *n* = 6). Area under the curve shown at right. All data are means ± SEM (Student's *t* test) from more than two independent experiments. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant.

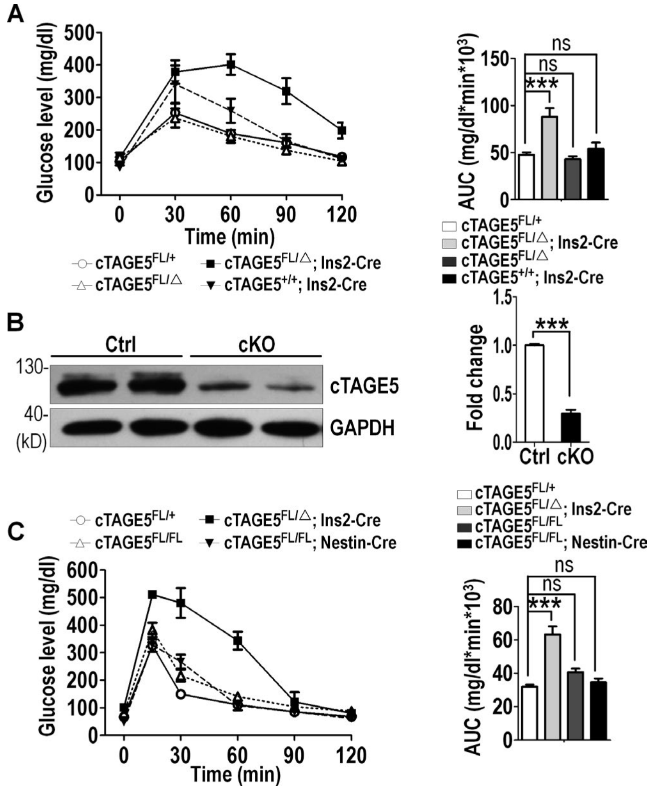

Figure S2. **Hyperglycemia is observed only in *cTAGE5*$^{FL/\Delta}$*; Ins2-Cre* mice.** (A) IPGTT was performed on 8-wk-old male mice of different genotypes (*cTAGE5*$^{FL/+}$, *cTAGE5*$^{FL/\Delta}$*; Ins2-Cre*, *cTAGE5*$^{+/+}$*; Ins2-Cre*, and *cTAGE5*$^{FL/\Delta}$; control [Ctrl] and cKO, *n* = 5). Area under the curve (AUC) shown at right. (B) Western blotting analysis of cTAGE5 expression in hypothalamus from 8-wk-old male mice. Quantitation of cTAGE5 protein levels normalized to GAPDH loading control shown at right. (C) IPGTT was performed on P18 male mice of different genotypes (*cTAGE5*$^{FL/+}$, *n* = 5; *cTAGE5*$^{FL/\Delta}$*; Ins2-Cre*, *n* = 4; *cTAGE5*$^{FL/FL}$, *n* = 7; and *cTAGE5*$^{FL/FL}$*; Nestin-Cre*, *n* = 5). Area under the curve shown at right. All data are means ± SEM (Student's *t* test) from three independent experiments. ***, P < 0.001; ns, not significant.

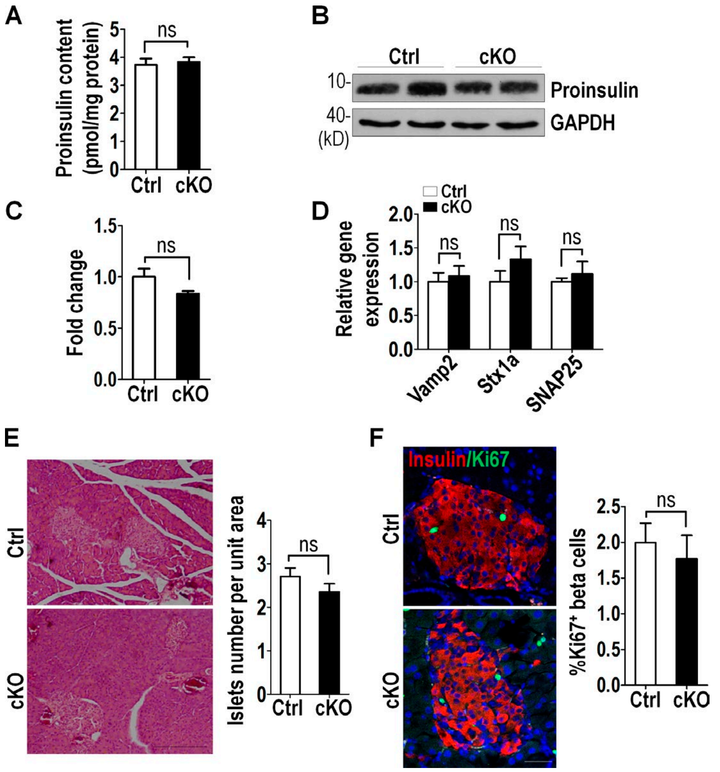

Figure S3. **The proinsulin levels, genes involved in exocytosis, and β cell proliferation are unchanged in cKO islets.** All data were from 8-wk-old male mice. (A) Total proinsulin content in isolated islets (control [Ctrl] and cKO, *n* = 4). (B and C) Western blotting analysis of proinsulin expression in islets (B). Quantitation of proinsulin protein levels were normalized to GAPDH loading control (C). (D) Relative *Vamp2*, *SNAP25*, and *Stx1a* mRNA expression in isolated islets detected by real-time PCR (*n* = 4). (E) H&E staining on pancreas serial sections (Ctrl and cKO, *n* = 4). Bar, 200 µm. Quantitation of the number of islets per unit area shown at right (Ctrl, *n* = 160; cKO, *n* = 145). (F) Pancreas sections stained with insulin (red) and Ki67 (green; Ctrl and cKO, *n* = 3). Bar, 100 µm. Quantitation of the percentage of Ki67-positive cells in insulin-positive cells shown at right. All data are means ± SEM (Student's *t* test) from more than two independent experiments. ns, not significant.

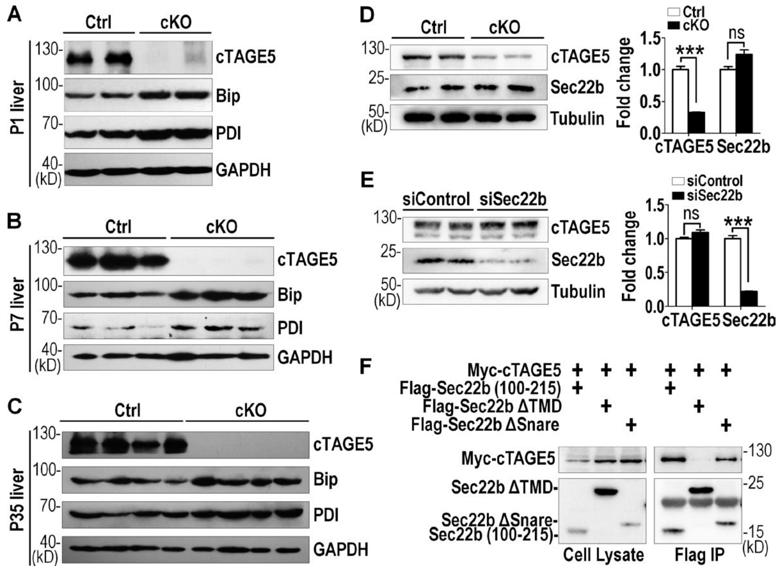

Figure S4. **Deletion of *cTAGE5* in the liver leads to ER stress, and cTAGE5 and Sec22b do not affect each other's expression.** (A–C) Western blotting analysis of cTAGE5, Bip, and PDI expression in P1 liver (A), P7 liver (B), and P35 liver (C). (D) Protein levels of Sec22b in control (Ctrl) and *cTAGE5* cKO islets from 8-wk-old male mice. (E) Protein levels of cTAGE5 were analyzed in control and Sec22b knockdown MIN6 cells. The relative protein levels were normalized to tubulin loading control. (F) Constructs expressing cTAGE5 and Sec22b mutants were transfected alone or in combination into 293 cells. 24 h later, cell lysates were precipitated with Flag agarose beads, and the immune complexes were detected. ΔTMD, transmembrane domain deleted. All data are means ± SEM (Student's *t* test) from three independent experiments. ***, P < 0.001; ns, not significant.

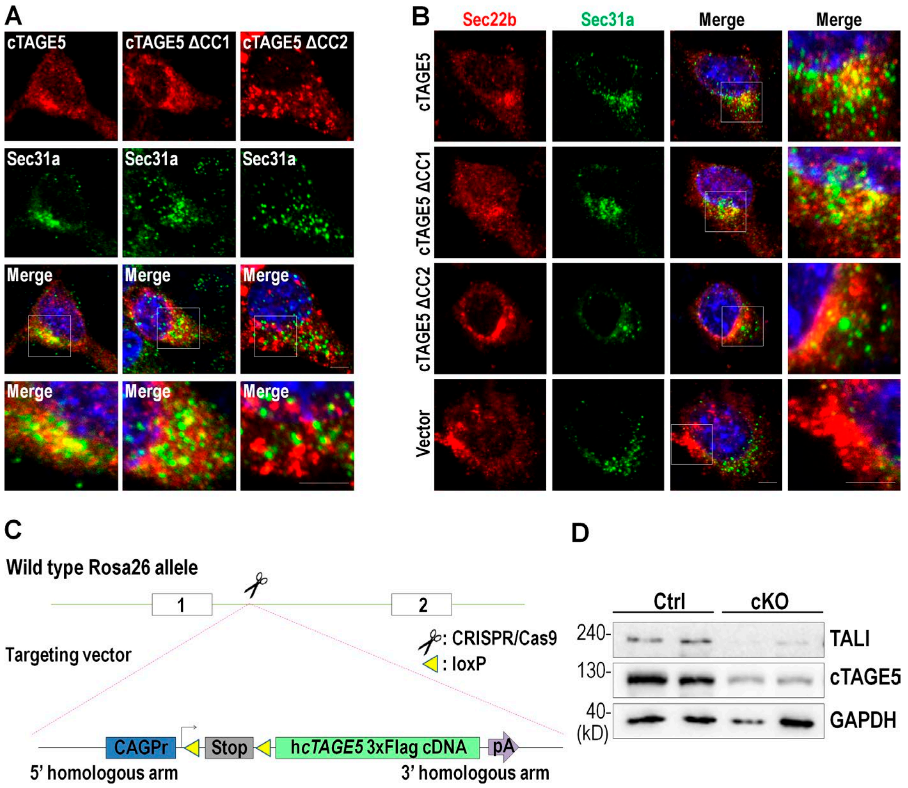

Figure S5. **The cellular location of cTAGE5 full-length or mutant and Sec22b in cTAGE5 knockdown MIN6 cells.** (A) cTAGE5 knockdown MIN6 cells were transfected with Flag- tagged cTAGE5 full-length, cTAGE5 △CC1, and cTAGE5 △CC2; 24 h later, cells were fixed with cold methanol and immunostained with Flag (red) and Sec31a (green). The bottom shows magnification of the indicated regions on the top. Data were from three independent experiments with similar results. Bar, 10 µm. (B) cTAGE5 knockdown MIN6 cells were transfected with cTAGE5 full-length, cTAGE5 △CC1, cTAGE5 △CC2, and vector; 24 h later, cells were fixed with cold methanol and immunostained with Sec22b (red) and Sec31a (green). The right shows magnification of the indicated regions on the left. Data were from three independent experiments with similar results. Bar, 10 µm. (C) Schematic representation of the human *cTAGE5* transgenic mice. Blue, CAG promoter; yellow, loxp site; gray, stop codon; green, hc*TAGE5* 3xFlag cDNA; purple, poly A tail. (D) Western blotting analysis of TALI and cTAGE5 expression in isolated islets from 8-wk-old male mice.

Table S1. **SYBR green primers for real-time PCR analysis**

Gene name	Sequence (5′ to 3′)
Ins1	F:AGCATCTTTGTGGTCCCCAC
	R:CCCCACACACCAGGTAAAGAG
Ins2	F:GCGTGGCTTCTTCTACACAC
	R:GTTCTCCAGCTGGTAGAGGG
cTAGE5	F:TTGGACATGAAGAGTGGCCTAGA
	R:AAGCGCCCTTTGGCTGAT
Bip	F:ACCCTTACTCGGGCCAAATT
	R:AGAGCGGAACAGGTCCATGT
Chop	F:CCAACAGAGGTCACACGCAC
	R:TGACTGGAATCTGGAGAGCGA
Vamp2	F:TGAGGTTCCCATCACCTCTC
	R:CTGTGGGGTTTGCTTTTGTT
Stx1a	F:GAACAAAGTTCGCTCCAAGC
	R:ATTCCTCACTGGTCGTGGTC
SNAP25	F:CAACGTGCAACAAAGATGCT
	R:GGGGGTGACTGACTCTGTGT
Actin	F:GCAAGTGCTTCTAGGCGGAC
	R:AAGAAAGGGTGTAAAACGCAGC

F, forward; R, reverse.