Supplement Table 1. The sequences of qPCR primers

Primers	**Sequence（5'-3'）**
cKO-F	GAGAATCTGGACTTGTTGCTGG
cKO-R	GCTGTGGTCTGAATTCTGGAAAAG
CASK-F	AAGGAGAAAACTAAAGGGTGC
CASK-R	GGAGGTAGGGTCTTCGGAG
PDX1-F	CCCCAGTTTACAAGCTCGCT
PDX1-R	CTCGGTTCCATTCGGGAAAGG
NeuroD-F	ATGACCAAATCATACAGCGAGAG
NeuroD-R	TCTGCCTCGTGTTCCTCGT
Mafa-F	AGGAGGAGGTCATCCGACTG
Mafa-R	CTTCTCGCTCTCCAGAATGTG
Mint1-F	GGTGCTGAGTCATCAAGCATAC
Mint1-R	GAACTTCAACGTAGGTTGGGAA
Munc 18-F	GTGGACCAGTTAAGCATGAGG
Munc 18-R	GCTCTCGGCGCTTGTTGAT
SNAP-25-F	CAACTGGAACGCATTGAGGAA
SNAP-25-R	GGCCACTACTCCATCCTGATTAT
Syntaxin-1-F	AGAGATCCGGGGCTTTATTGA
Syntaxin-1-R	AATGCTCTTTAGCTTGGAGCG
VAMP-F	GCTGGATGACCGTGCAGAT
VAMP-R	GATGGCGCAGATCACTCCC

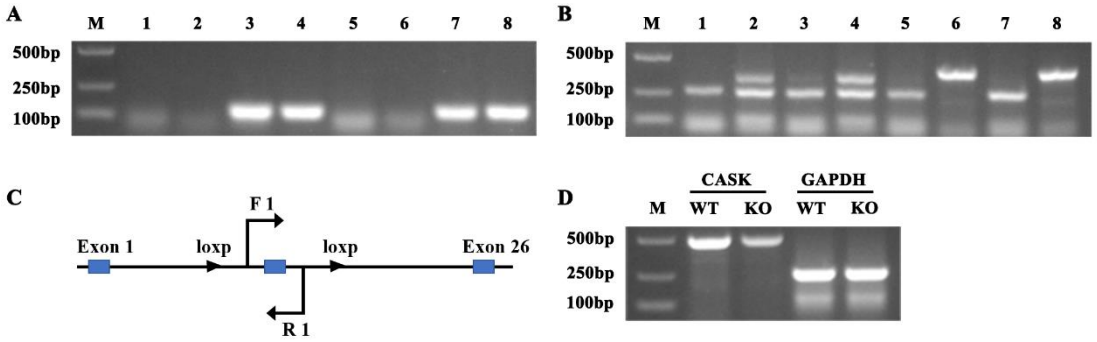
GAPDH-F	AGGTCGGTGTGAACGGATTTG
GAPDH-R	TGTAGACCATGTAGTTGAGGTCA

Notes: cKO: the primers were designed within exon 6 of *Cask* gene.

Supplementary Table 2. Antibodies for western blot assay used in the experiments.

Name	Manufacturer	Dilution
Anti-CASK	Santa Cruz Biotechnology, Santa Cruz, CA, USA	1:1000
Anti-IRS1	Cell Signaling Technology, Danvers, MA, USA	1:1000
Anti-phospho-IRS1 (Ser 636)	Cell Signaling Technology, Danvers, MA, USA	1:1000
Anti-AKT	Cell Signaling Technology, Danvers, MA, USA	1:1000
Anti-phospho-AKT (Ser 473)	Cell Signaling Technology, Danvers, MA, USA	1:2000
Anti-mTOR	Abcam, Cambridge, UK	1:1000
Anti-phospho-mTOR (Ser2448)	Abcam, Cambridge, UK	1:1000
Anti-p70S6K	Abcam, Cambridge, UK	1:1000
Anti-phospho-p70S6K (Ser 424)	Abcam, Cambridge, UK	1:1000
Anti-GAPDH	Cell Signaling Technology, Danvers, MA, USA	1:1000
Anti-β-Actin	Cell Signaling Technology, Danvers, MA, USA	1:1000

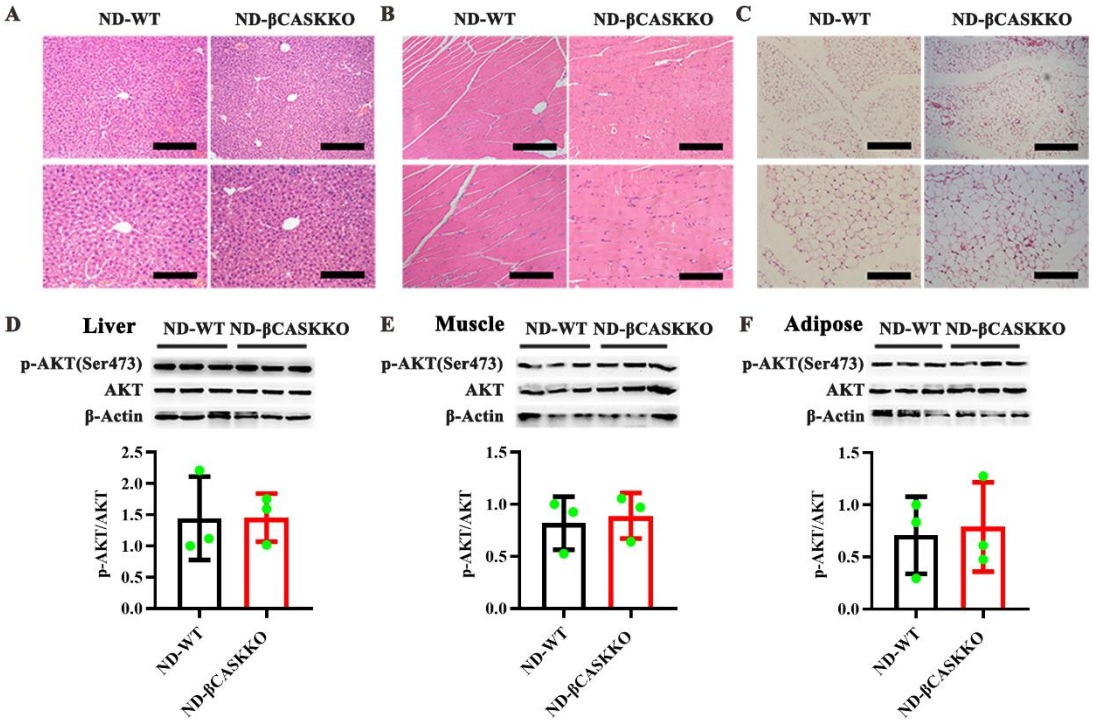
Supplementary Figure 1



Representative PCR results for the genotyping identification

(A-B) Identification of the Cre recombinase transgene and LoxP sites in mice. **(A)** Lanes 1, 2, 5, 6: mice did not carry the Cre recombinase transgene; Lanes 3, 4, 7, 8: mice carried the Cre recombinase transgene. **(B)** Lanes 1,3,5,7: mice did not insert the LoxP site; Lanes 2,4: $CASK^{fl/-}$ heterozygous mice; Lanes 6,8: $CASK^{fl/fl}$ mice. **(C)** Identification of the efficient removal of exon 6 in CASK gene of mouse pancreatic islets by PCR assay. Primers were designed to recognize the region inside of LoxP sites and both sides of exon 6. The primers for the *Cask* as follow: F1: GTAGGAGTCTCCACTTTGTGAGTC; R1: CTTCAGAGTCTCCGGGTAGTTTTC. The primers for the *Gapdh* as follow: F: GGAGAGATCTGGTTTCTGGAGGATG; R: CCCCAGAGGTAGTTATGGCGTAGTG. **(D)** The results showed that in comparison with WT mice, the PCR products (exon 6 in *Cask* gene) in the islets of βCASKKO mice were significantly decreased. The residual bands should be from non-β cells in the islets.

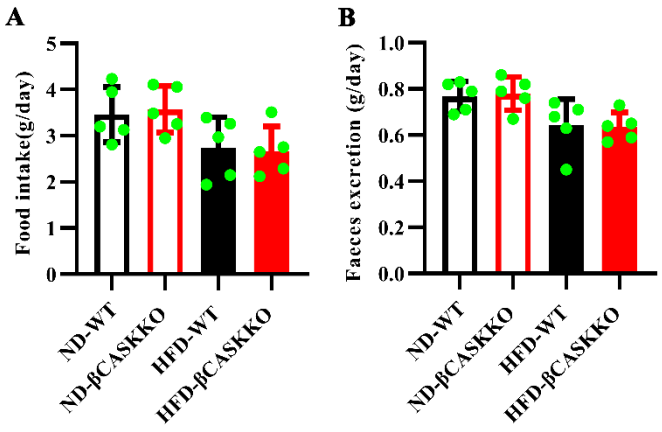
Supplementary Figure 2



ND-βCASKKO mice do not show effects on insulin signaling in liver, skeletal muscle, and epididymal adipose tissue

(A-C) Representative H&E staining of the liver **(A)**, skeletal muscle **(B),** and epididymal adipose tissue **(C)** from ND-WT and ND-βCASKKO mice. Scale bars: (upper) 200 μm; (lower) 100 μm. **(D-F)** Western blot analysis of the phosphorylation level of AKT in the liver **(D)**, skeletal muscle **(E),** and epididymal adipose tissue **(F)** of mice after insulin treatment. Protein levels were normalized to the β-actin level. The intensity of the protein bands is indicated below the respective bands. n=3.

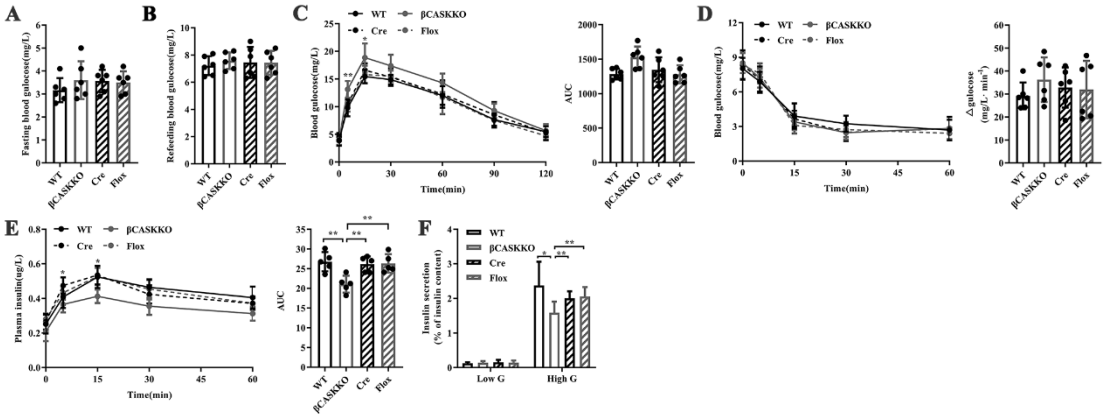
Supplementary Figure 3



Metabolic cage study of mice after HFD for 16 weeks

(A-B) The average day food intake **(A)** and feces excretion **(B)** of mice after ND or HFD feeding for 16 weeks. n=5.

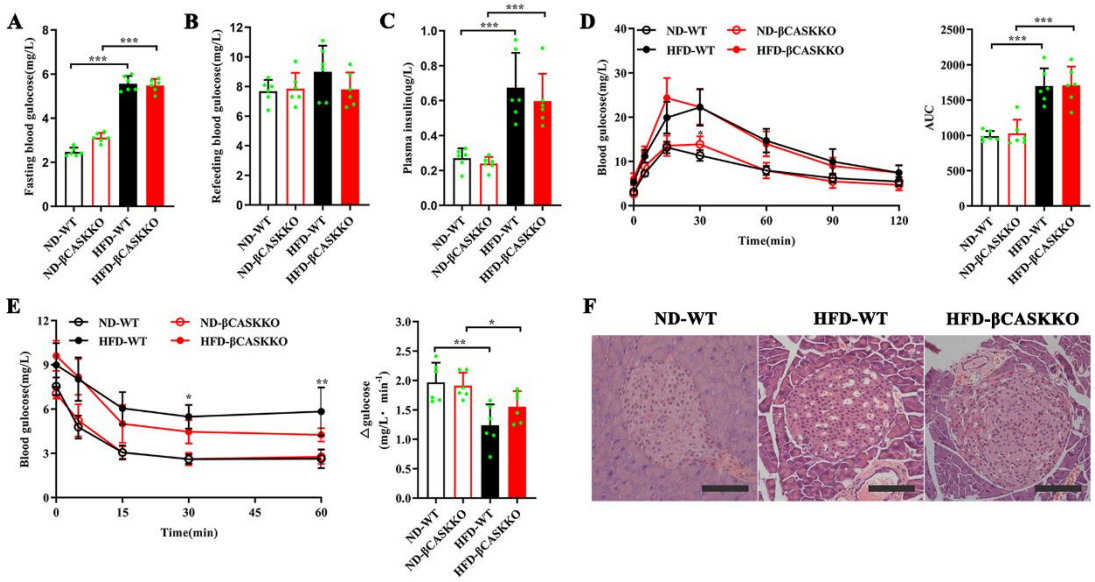
Supplementary Figure 4



Glucose metabolism phenotype in female mice

(A-B) Fasting **(A)** and postprandial **(B)** blood glucose levels of βCASKKO and WT mice. **(C)** IPGTT assays were performed on 16-week-old mice. The AUC is shown on the right. n=6, * P<0.05, ** P<0.01. **(D)** IPITT assays were performed on 16-week-old mice. The rate of decline in glucose between 0 and 15 min is shown on the right. n=6. **(E)** GSIS assays were performed in mice. n=5, * P<0.05, ** P<0.01. AUC is shown on the right. **(F)** Pancreatic islets were separated to perform a GSIS. Insulin release in response to glucose stimulation was calculated as a percentage of insulin content. n=8. * P<0.05, ** P<0.01. These results showed that βCASKKO mice decreased insulin secretion upon glucose stimulation.

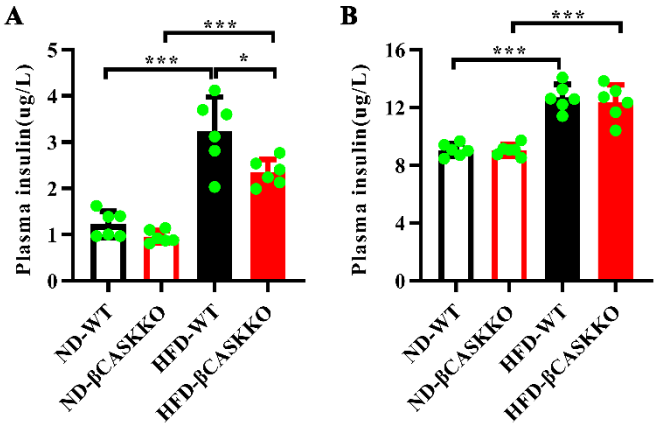
Supplementary Figure 5



Glucose metabolism phenotype in HFD mice fed the HFD for 8 weeks

(A-C) Fasting **(A)** and postprandial **(B)** blood glucose levels and fasting serum insulin level **(C)** of WT and βCASKKO mice for HFD and ND. n=6. ***P<0.001. **(D-E)** Glucose tolerance test **(D)** and insulin tolerance test **(E)** assays were conducted in HFD and ND mice after 8 weeks of ND or HFD feeding. n=6. * P<0.05, ** P<0.01, WT vs. βCASKKO. The AUC or the rate of decline in glucose between 0 and 15 min is shown on the right side of the corresponding curve. * P<0.05, ** P<0.01, *** P<0.001. **(F)** Representative H&E images of the pancreas showed enlarged islets in HFD mice. Scale bars: 100 μm. These results showed that after 8 weeks of HFD feeding, HFD-WT mice and HFD-βCASKKO mice showed less difference in glucose homeostasis and insulin sensitivity.

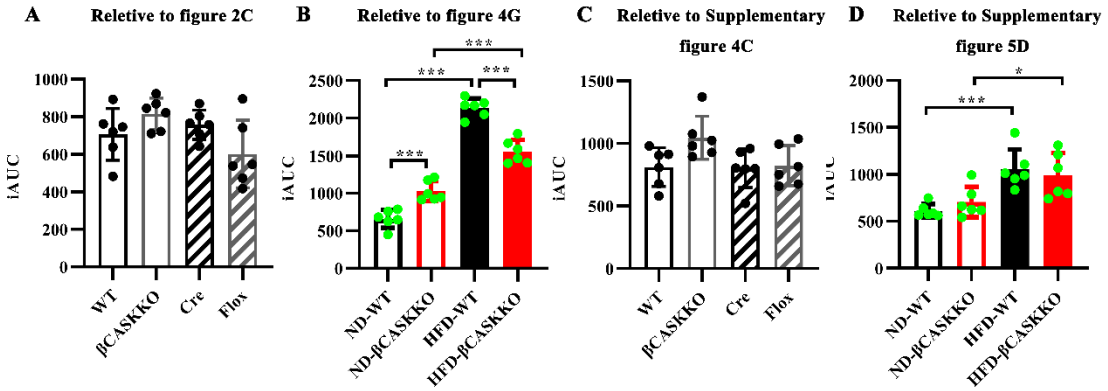
Supplementary Figure 6



Determination of serum insulin levels before and after injection of 1 U insulin/kg body weight in 4 h fasted mice during insulin tolerance test

(A-B) Serum insulin level in fasted **(A)** or 15 min **(B)** after insulin injection. n=6, $^{**}P<0.005$, $^{***}P<0.001$. These results showed that the circulating insulin level 15 min after insulin injection between WT and KO mice was approximately equal in both ND and HFD-treated group.

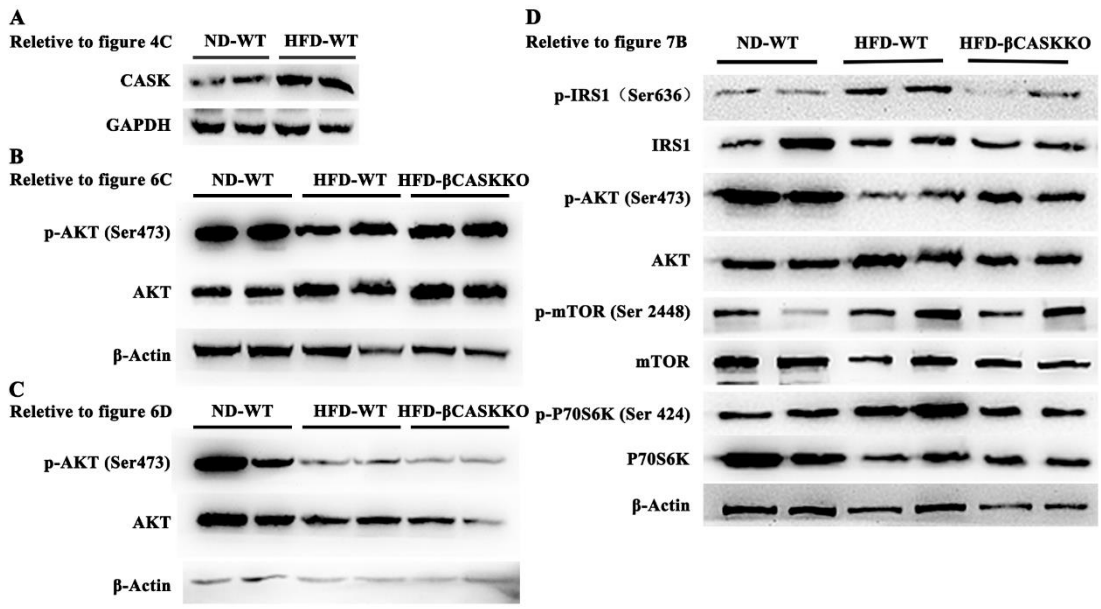
Supplementary Figure 7



The iAUC values for IPGTT

(A-C) The iAUC related to **Figure 2C (A)**, **Figure 4G (B)**, **Supplementary Figure 4C (C)**, and **Supplementary Figure 5D.**

Supplementary Figure 8



Western blot analyses of protein

(A) Western blot analyses of CASK and GAPDH in islets from ND-WT and HFD-WT mice, related to **Figure 4C** in the article. n=4 mice/group. **(B-C)** Western blot analyses of the phosphorylation level of AKT in the liver **(B)** and muscle tissue **(C)** of mice 15 min after insulin treatment, related to **Figure 6C-D** in the manuscript, respectively. **(D)** Western blot analyses of the phosphorylation level of IRS1, AKT, mTOR, and S6K1 in the adipose of mice 15 min after insulin treatment, related to **Figure 7B** in the manuscript.