

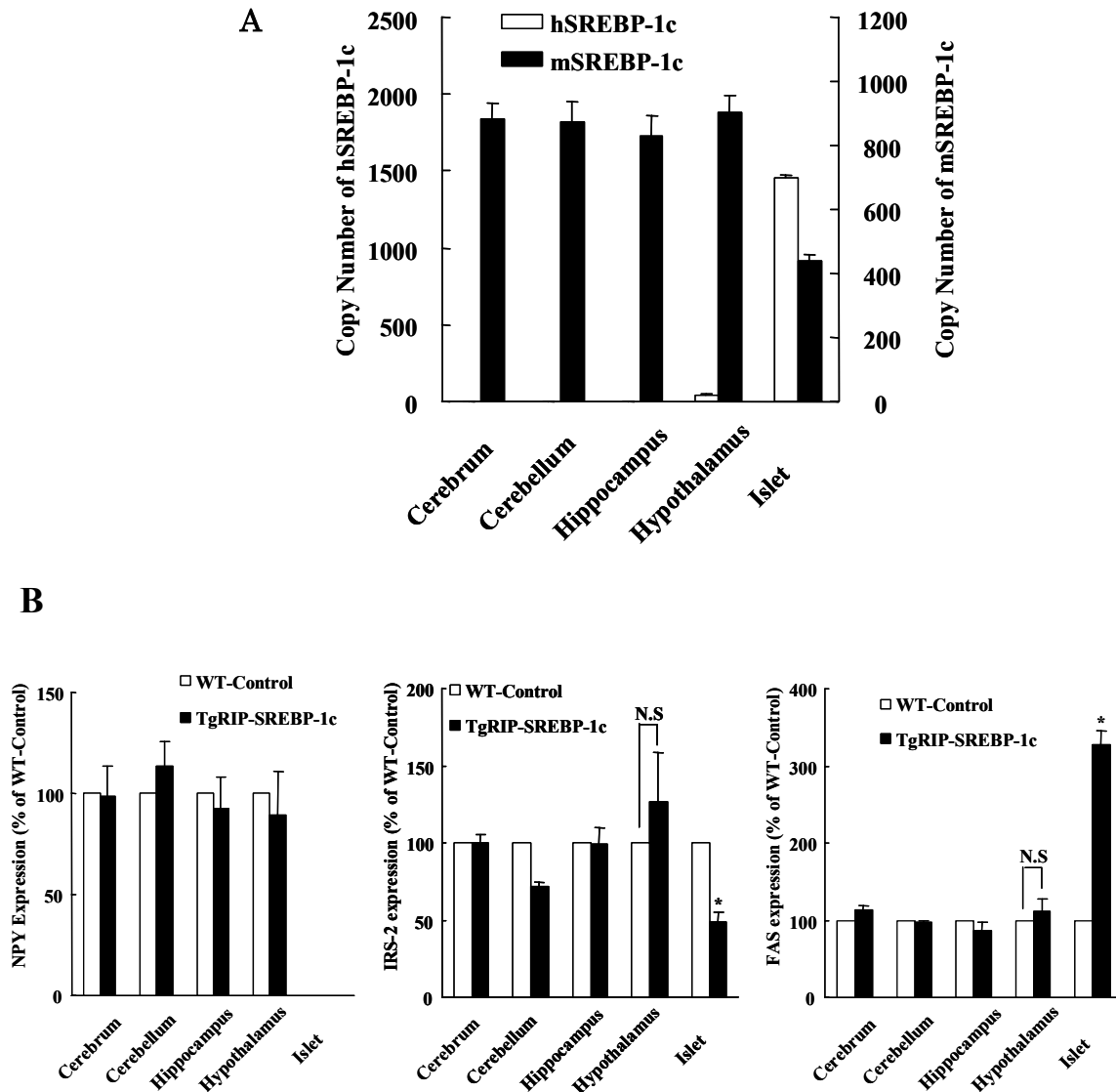
Supplemental data

Granuphilin is activated by SREBP-1c and involved in impaired insulin secretion in diabetic mice

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Supplemental figures

Figure S1



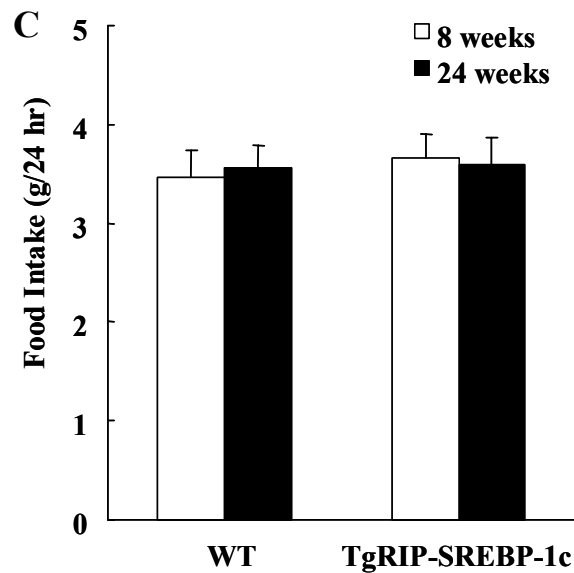


Figure S1 Transgene (human nuclear SREBP-1c) expression in brain and pancreatic islets of transgenic mice under rat insulin promoter I

(A) mRNA levels of the human and mouse SREBP-1c on brain fractions and pancreatic islets from male age-matched (15-18 weeks-old) WT-Control or TgRIP-SREBP-1c mice as estimated by real-time PCR. hSREBP-1c levels were calculated as copy numbers. (B) mRNA levels of NPY, IRS-2, and FAS on the indicated tissues from the indicated mice as estimated by real-time PCR. * $p < 0.01$ (vs WT-Control mice). N.D; not detectable. (C) Analysis of food intake from male 8 weeks-old or 24 weeks-old mice over 24 hrs using metabolic cage system. Results are expressed by mean \pm SEM. Studies were performed in triplicate from three to four mice per replicate.

Figure S2

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-1190      -1180      -1170      -1160      -1150      -1140
TAGCCGTTGA GAATGAAATG AGAGAGAGA GAGTGAGAAA TTGCAGACAT GTCTCACTG
                HNF-3β

-1130      -1120      -1110      -1100      -1090      -1080
TAAAATAGTT TCATATAATT GACCCCTAAA GGAGGTATAG AGAGATCTTG CATATGGGTA
                HNF-3β

-1070      -1060      -1050      -1040      -1030      -1020
GGACCCAGAT TTCTTTTAA GATGGTAGAA AGTTTAGTAC GTTTCATGGG TAGCCAGTAGA

-1010      -1000      -990      -980      -970      -960
TAGACCGGAT CTGCCCTACTT TTTACTGCTG ACGTGAAGCA AAGACAGAGA GGAAGGTCGA
                CREB

-950      -940      -930      -920      -910      -900
GATCTGGGTA ATATAAACA TTTTGTGTG TATCGAAGA CAATGAGCAC ATGCTTTCAA
                HNF-3β

-890      -880      -870      -860      -850      -840
AAGACAGTTC TTAAGCAGA TCCGTGCCCTG CTAGAAGTGA CAGAAGAGCT GACCTGGCGC

-830      -820      -810      -800      -790      -780
TTCCCTTAGC CTTTTACAAC CCACGCTTGT TCTCAATTGT TGTCTAGTTT TTAATGAGGT

-770      -760      -750      -740      -730      -720
GACTGATTTA ACTAGTTGTG TCTTTGAGTG TGAATGCATG AAATTAATCT ATACCCCTTC

-710      -700      -690      -680      -670      -660
CCTAAGAGTG ACTTTTACTT GCATCATAAA AACAAAGAGT CAATCTGAAT GATATATGTG
                HNF-3β

-650      -640      -630      -620      -610      -600
TTAATATTC CCAAATAAA ATTGAGACCA TTCCCTCTCC TGTTCCTCTA CCACTGGCCC
PDX-1                HNF-1

-590      -580      -570      -560      -550      -540
TGCCCTGTCT TCCTTTCCCC ACACCCCAT ATAGTCATCT TTCTATCTTT TTTAAAAGTT

-530      -520      -510      -500      -490      -480
TTTACAGATT ATTATTTCC ATGACCCAA CCGTTTCATC AGGCTTGCTT ACAGGGCGAT
                HNF-1

-470      -460      -450      -440      -430      -420
TATTTACAGG CACTGGGCT CCTTACCAT ACCTACATCA ATGAAGAAGT TATCTTTCTC

-410      -400      -390      -380      -370      -360
TCTCCCTCC CCCCGGCCA GTGACTGACT CTTGTTTTCT TTTCTTTCT TTTTTTAA
                IRE

-350      -340      -330      -320      -310      -300
TTAGATATTT TCTCATTTA CAITTCAAA GAAGTACTTT ACTATTTGGC AACTTTTGGC

-290      -280      -270      -260      -250      -240
TGCCTGAACA AAAAGCCAG TTAACTTTAT TCCCATTCCT ACCCCCACC CCGACCCCCA
                E-box ERE                ERE
                -----
                HNF-4

-230      -220      -210      -200      -190      -180
TCATCTAGA GAGTGAGCA AGATAGACTA GACGGAAATG GCGAGGAGG GACATCCAGT

-170      -160      -150      -140      -130      -120
AGGTCTCAA ACTCTCTAA CTCAGGAGGA AATTCTTCCA GTGCCPTAAG GCTTTGGTTC
                MARE

-110      -100      -90      -80      -70      -60
CCAGAATCT CTACTGTACT AGTTGGCCCT CTGCTCAAAT AGCCCTGCCG TCAGTGCGGC

-50      -40      -30      -20      -10      0
CCCTTTAAGA GTCCAGCTCC TCCCGCTCC AGGAAAGTT GCGCTTTTTT CCGCTCTCTG

*
10          20          30          40          50          60
GGAGGTTGA GTAGGCGCGG CCGCCTGGGT TGCTGGTCTA AGTCTTTTTG GAGCTAGAGA

70          80          90          100         110         120
CCCTTCAAAC CATTCGGCAC CCGGACCTTT GGTCACTTAG TAGCACTGT TGCAGGTGAC

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Figure S2 DNA sequences of mouse granophilin promoter

Mouse granophilin promoter sequences. The consensus sequences of transcriptional

factors are underlined (dotted: potential consensus).

Figure S3

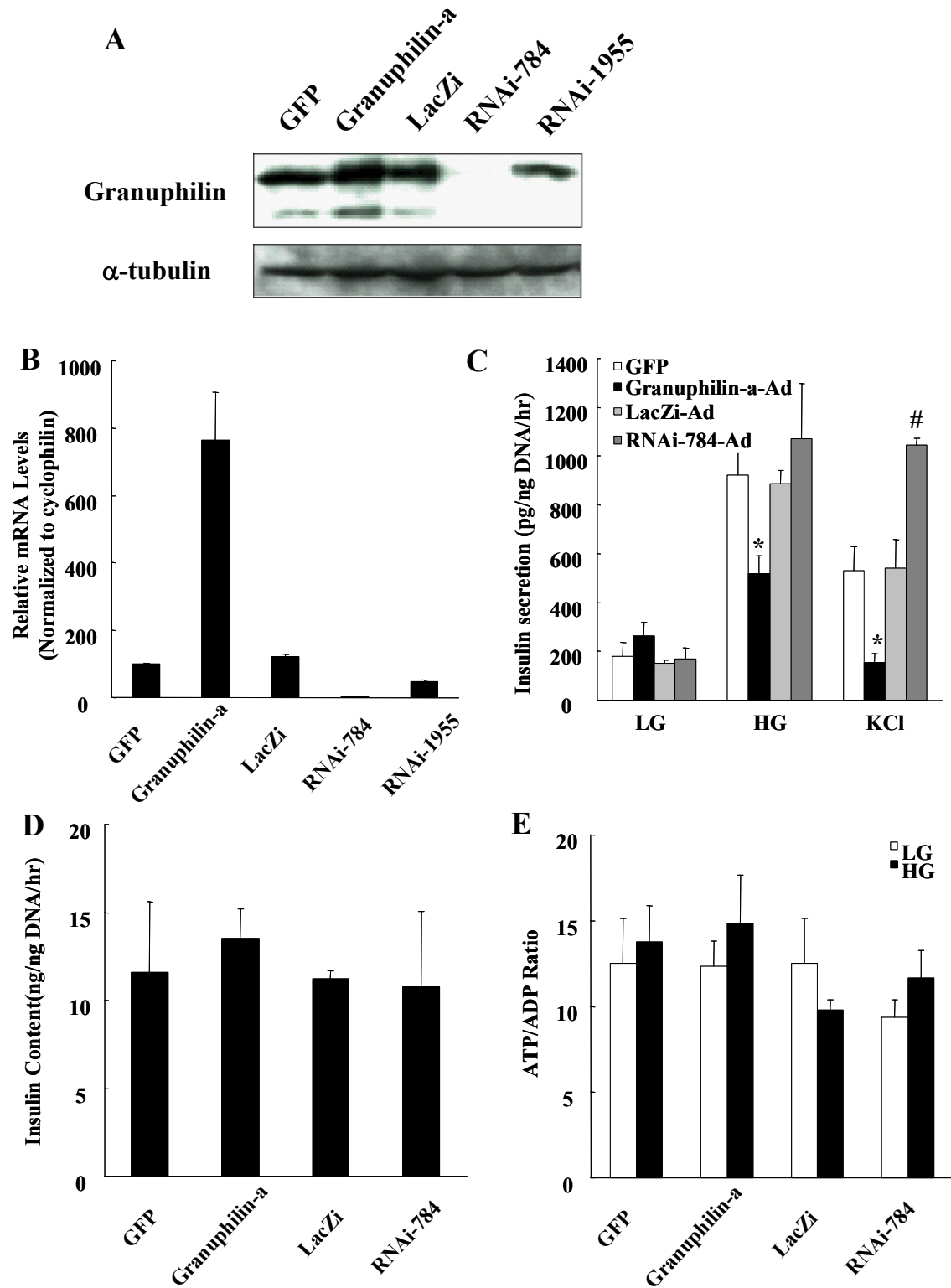


Figure S3 Effects of adenovirus-mediated over-expression and knockdown of granuphilin on insulin secretion in murine pancreatic islets

Islets were isolated from male C57BL/6 mice (10 weeks-old) and were infected with adenoviral-GFP, -granuphilin-a or -granuphilin-RNAi (RNAi-784, -1955) (500 MOI) for 48 hrs. (A) Immunoblot analysis on total proteins of the islets with antibody against mouse granuphilin, and α -tubulin as a loading control. (B) mRNA level of granuphilin from the islets as estimated by real-time PCR. (C) LG-, HG-, and KCl-stimulated insulin secretion in the islets treated with adenoviral-GFP (white bars), -granuphilin-a (black bars), -LacZ-RNAi (light grey bars), and -granuphilin-RNAi-784 (dark grey bars). * $p < 0.05$ (vs GFP) and # $p < 0.05$ (vs LacZi). (D) Cellular insulin contents from the islets. (E) Cellular ATP/ADP ratio from the islets. Results were normalized to cellular DNA content (C and D) and are expressed by mean \pm SEM. Studies were performed in triplicate with sets of islets pooled from three to four mice per replicate.

Figure S4

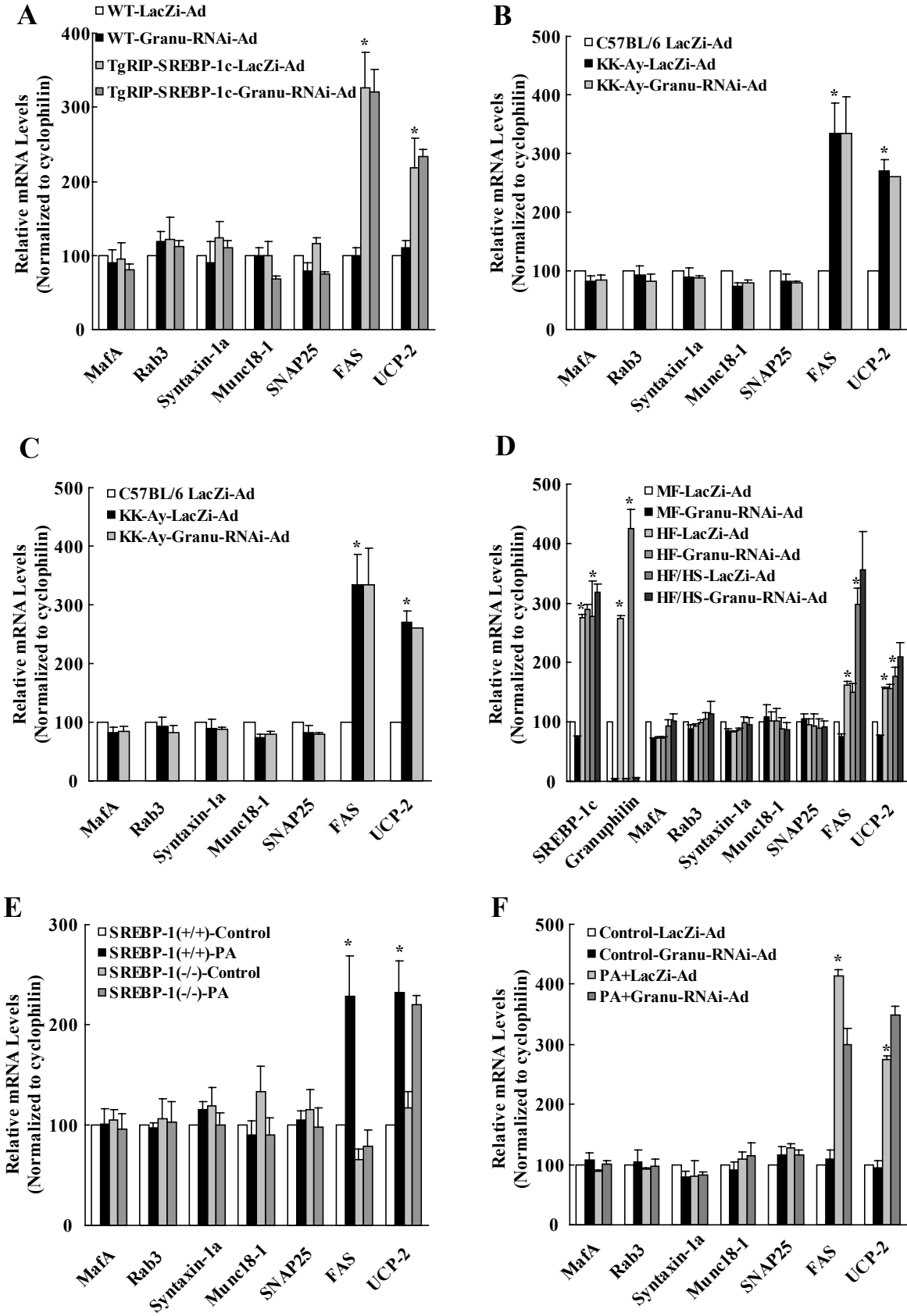


Figure S4 Gene expression profiles

(A) mRNA levels of the fusion machinery for exocytosis of insulin granules and SREBP-1c target genes from WT-Control or TgRIP-SREBP-1c mice islets as estimated by real-time PCR. * $p < 0.01$ (vs WT-LacZi). (B) mRNA levels of indicated genes from C57BL/6 or KK-Ay mice islets as estimated by real-time PCR. * $p < 0.01$ (vs C57BL/6-LacZi). (C) mRNA levels of indicated genes from C57BL/6 or ob/ob mice islets as estimated by real-time PCR. * $p < 0.01$ (vs C57BL/6-LacZi). (D) mRNA levels of indicated genes from diet-induced obese (DIO) mice islets as estimated by real-time PCR. * $p < 0.01$ (vs MF-LacZi). (E) mRNA levels of indicated genes from SREBP-1-null (SREBP-1(-/-)) and wild-type littermate (SREBP-1 (+/+)) mice islets as estimated by real-time PCR. * $p < 0.01$ (vs wild-type littermate (SREBP-1 (+/+))-Control). (F) mRNA levels of indicated genes from PA-treated or control islets as estimated by real-time PCR. * $p < 0.01$ (vs Control-LacZi). Each islets were infected with adenoviral-LacZ-RNAi (LacZi) or -granuphilin-RNAi (Granu-RNAi) (500 MOI) for 48 hrs. Results are expressed by mean \pm SEM. Studies were performed in triplicate with sets of islets pooled from three to four mice per replicate.

Figure S5

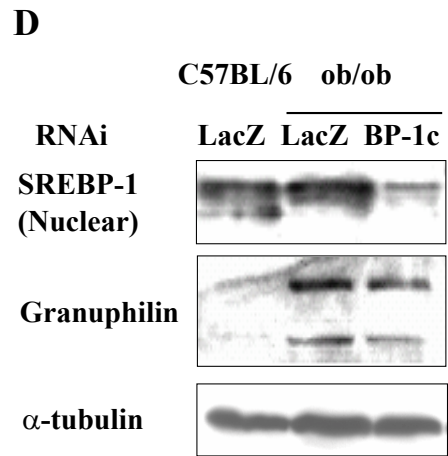
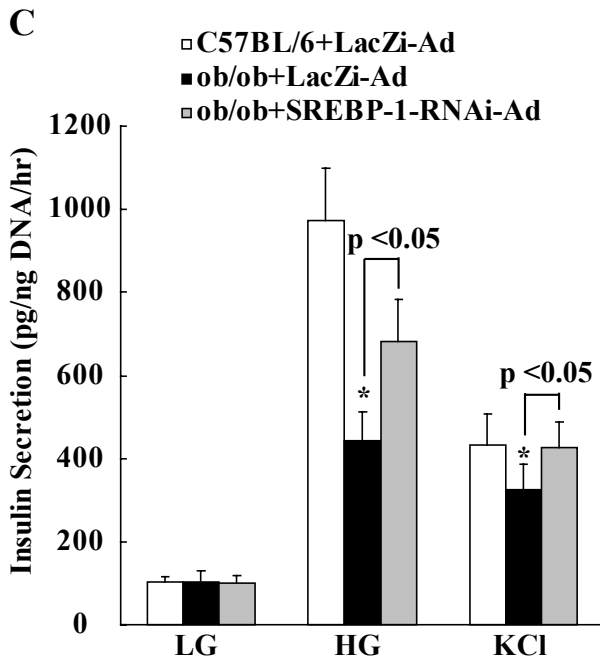
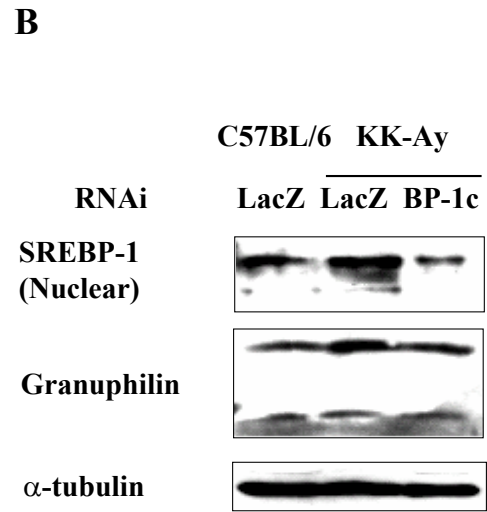
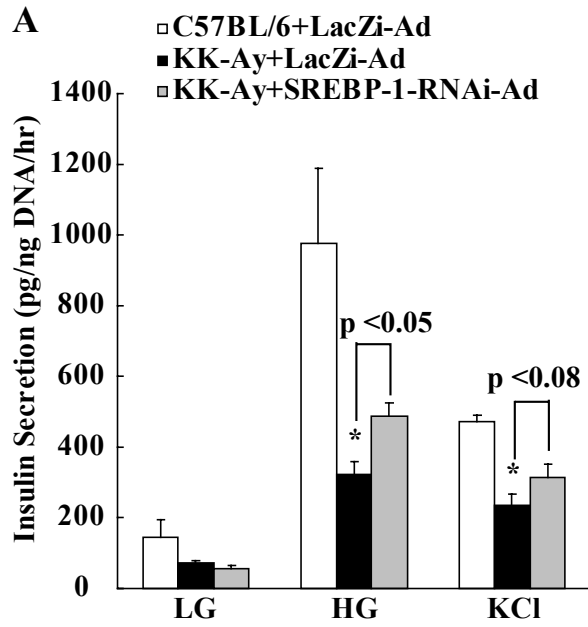


Figure S5 Effects of knockdown of SREBP-1 on insulin secretion in pancreatic islets from different diabetic model mice

Islets were isolated from male C57BL/6 mice, KK-Ay or ob/ob mice (10 week-old) and were infected with adenoviral-LacZ RNAi (LacZi-Ad) or SREBP-1 RNAi (500 MOI) for 48 hrs. (A) LG-, HG-, and KCl-stimulated insulin secretion in the indicated islets; C57BL/6-LacZi (white bars), KK-Ay-LacZi (black bars), KK-Ay-SREBP-1-RNAi (grey bars). * $p < 0.05$ (vs C57BL/6-LacZi). (B) Immunoblot analysis of SREBP-1 and mouse granuphilin from the indicated islets with α -tubulin as a loading control. (C) LG-, HG-, and KCl-stimulated insulin secretion in the indicated islets; C57BL/6-LacZi (white bars), ob/ob-LacZi (black bars), ob/ob-SREBP-1-RNAi (grey bars). * $p < 0.05$ (vs C57BL/6-LacZi). (D) Immunoblot analysis of SREBP-1 and mouse granuphilin from the indicated islets with α -tubulin as a loading control. Results were normalized to cellular DNA content (A and C) and are expressed by mean \pm SEM. Studies were performed in triplicate with sets of islets pooled from three to four mice per replicate.