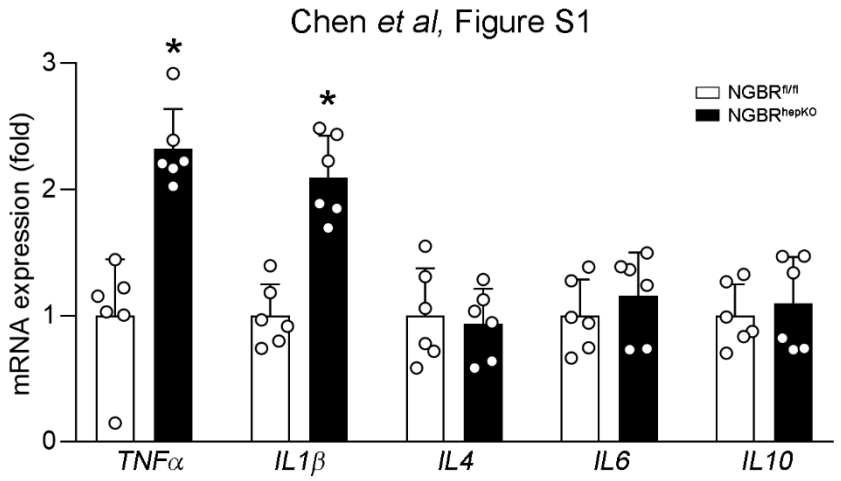

Figure S1. Reduced NGBR expression in the liver is associated with increased inflammatory molecules. Liver samples were collected from NGBR$^{fl/fl}$ and NGBR^{hepKO} mice and used to determine expression of mRNA for tumor necrosis factor α (*TNFα*), interleukin 1β, 4, 6, 10 (*IL1β, 4, 6, 10*) by qPCR. *, p<0.05 *versus* NGBR$^{fl/fl}$, n=6.

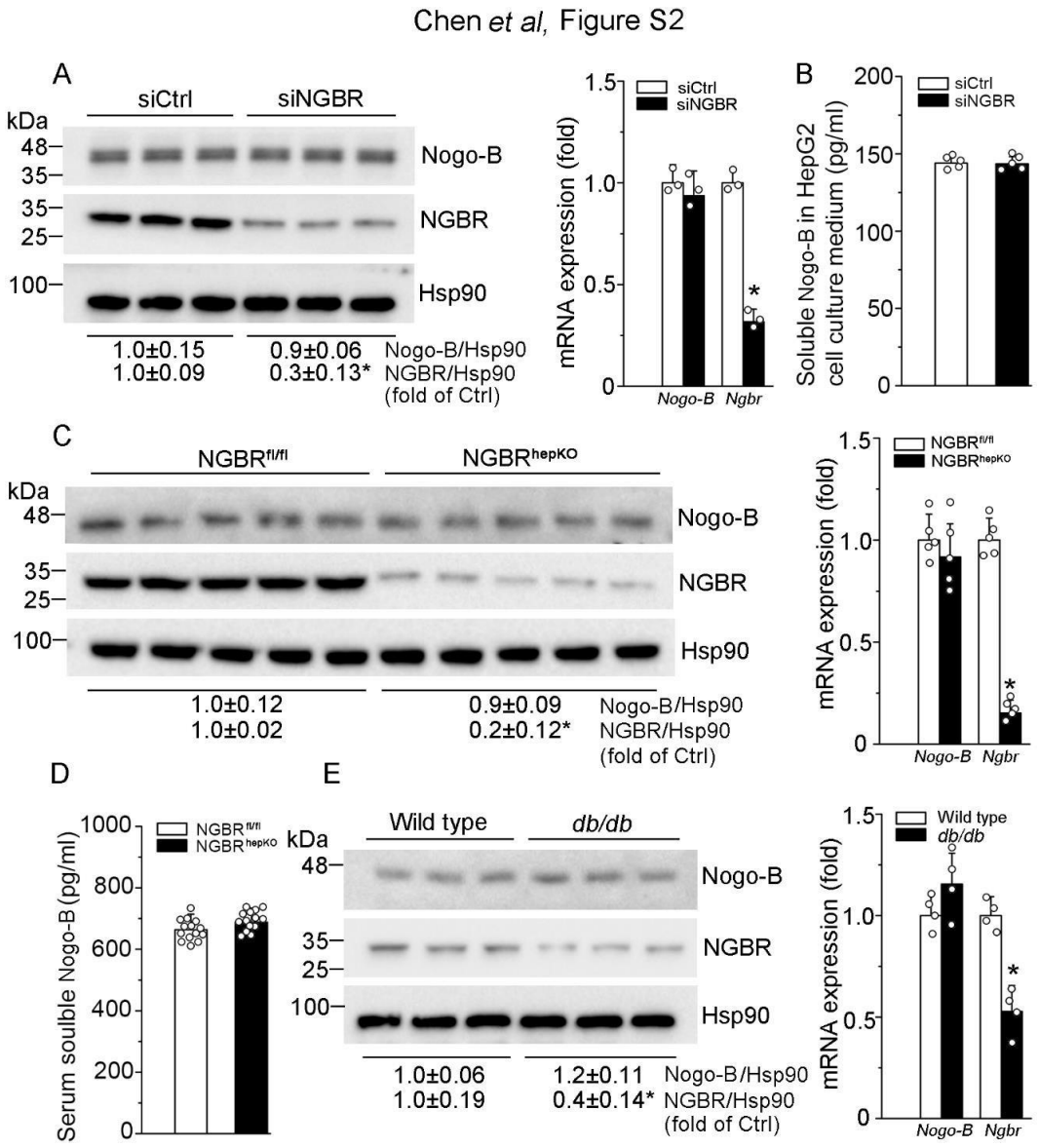

Figure S2. NGBR deficiency does not affect hepatic Nogo-B protein expression and secretion *in vitro* and *in vivo*. HepG2 cells in 6-well plates were transfected with scrambled siRNA (siCtrl, 50 nM) or NGBR siRNA (siNGBR, 50 nM) for 24 h. (A) Expression of Nogo-B and NGBR protein and mRNA were determined by western blot and qPCR, respectively. *, $p<0.05$, $n=3$. (B) Nogo-B content in HepG2 cell culture medium was determined by a human Nogo-B ELISA kit. $n=5$. (C) Expression of Nogo-B and NGBR protein and mRNA in livers of $NGBR^{fl/fl}$ and $NGBR^{hepKO}$ mice were determined by western blot and qPCR, respectively. *, $p<0.05$, $n=5$. (D) Serum Nogo-B levels of $NGBR^{fl/fl}$ and $NGBR^{hepKO}$ mice were determined by a mouse

Nogo-B ELISA kit. n=14. (E) Protein and mRNA expression of Nogo-B and NGBR in livers of wild type and *db/db* mice was determined by western blot and qPCR. *, p<0.05, n=4.

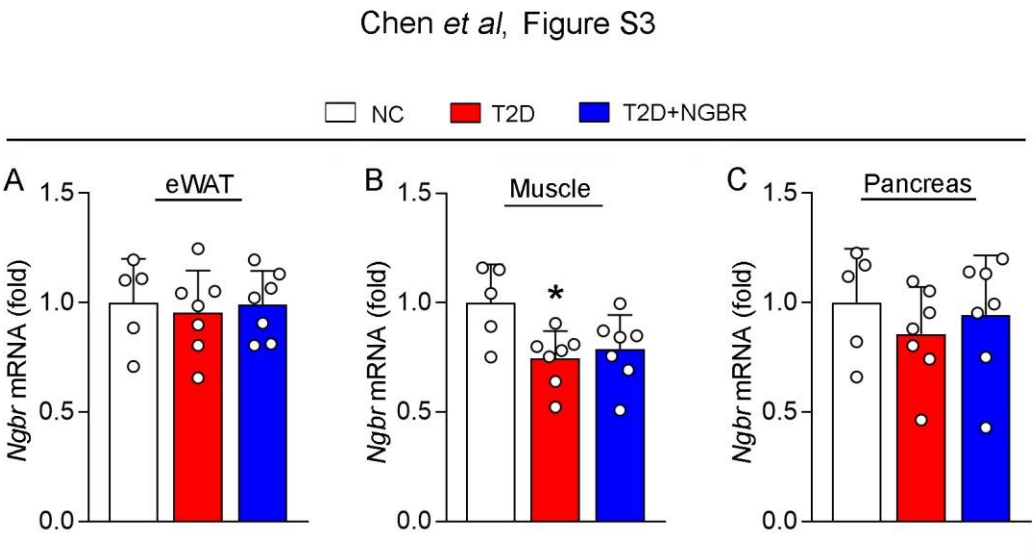

Figure S3. Determination of NGBR mRNA expression in mouse tissues. Samples of epididymal white adipose tissue (eWAT) (A), skeletal muscle (B) and pancreas (C) were collected from NC, T2D and T2D+NGBR mice used in Figure 2A, followed by preparation of tissue total RNA and determination of *Ngbr* mRNA expression by qPCR. *, $p<0.05$ *versus* NC, $n=5$ (NC group), $n=7$ (T2D or T2D+NGBR group).

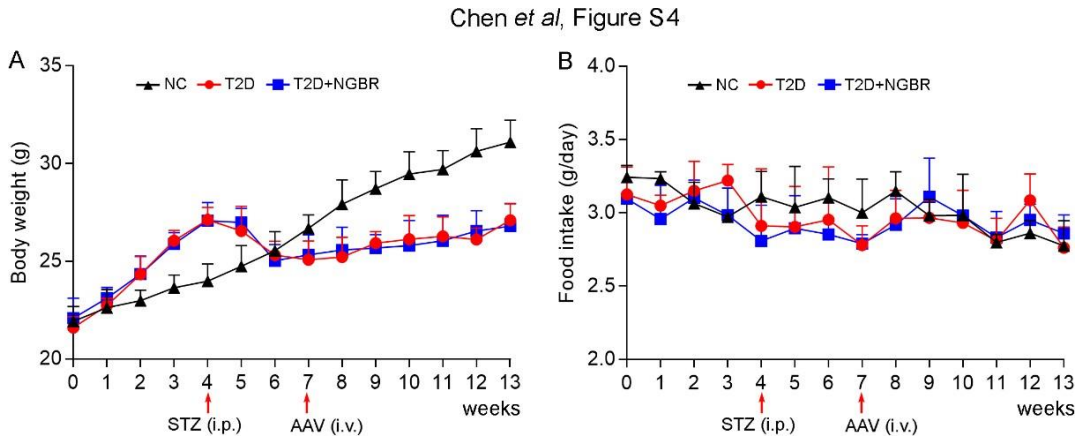

Figure S4. Injection of AAV-NGBR has little effect on bodyweight gain and food intake in high-fat diet (HFD)/streptozotocin (STZ) mice. (A, B) The experimental design was shown in Figure 2A. During this course, mouse bodyweight (A) and 24-h food intake (B) were monitored weekly. *n*=5 (NC group), *n*=7 (T2D or T2D+NGBR group).

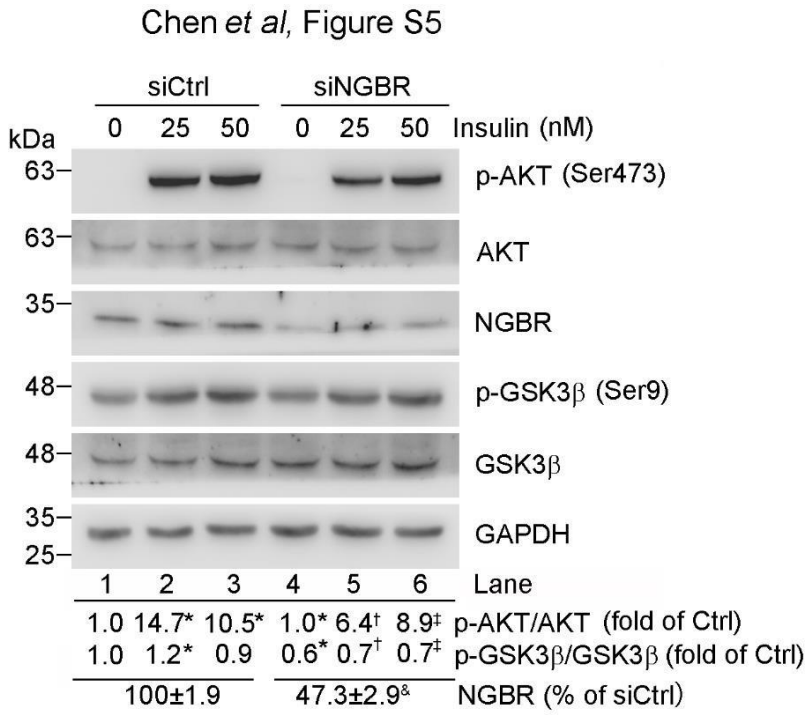

Figure S5. NGBR regulates insulin sensitivity through insulin signaling and AKT axis in primary hepatocytes. Primary hepatocytes isolated from wild type mouse liver were cultured in 6-well plates and transfected with scrambled siRNA (siCtrl, 50 nM) or NGBR siRNA (siNGBR, 50 nM) for 24 h. Cells were then treated with insulin at the indicated concentrations for 30 min. Expression of indicated proteins was determined by western blot. *, $p<0.05$ *versus* lane 1; †, $p<0.05$ *versus* lane 2; ‡, $p<0.05$ *versus* lane 3; &, $p<0.05$ *versus* siCtrl. $n=3$.

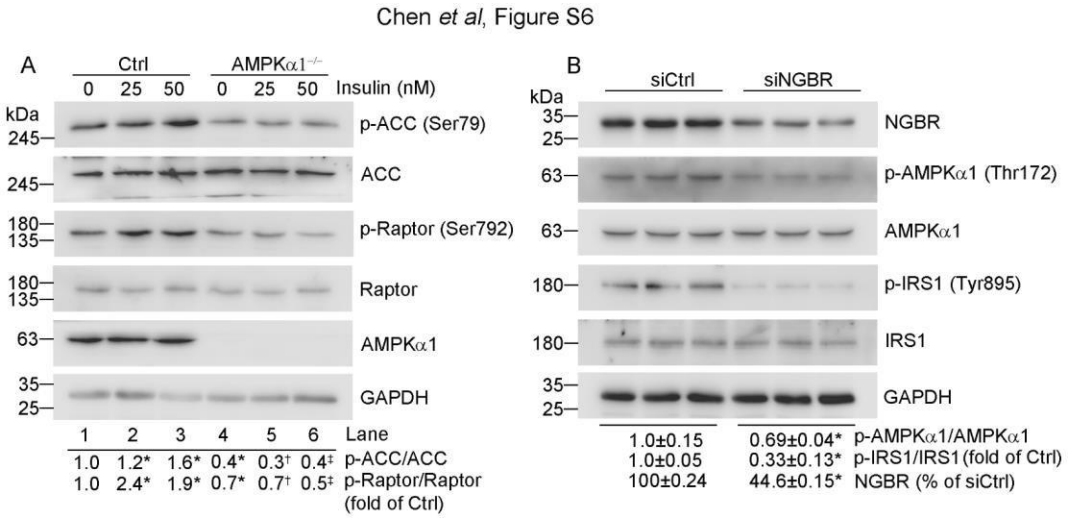

Figure S6. Reduced NGBR expression in HepG2 cells is associated with decreased phosphorylation of AMPK and insulin receptor substrate 1 (IRS1). (A) HepG2-Ctrl cells and HepG2-AMPK$\alpha 1^{-/-}$ cells in 6-well plates were treated with insulin at the indicated concentrations for 30 min. (B) HepG2 cells in 6-well plates were transfected with scrambled siRNA (siCtrl, 50 nM) or NGBR siRNA (siNGBR, 50 nM) for 24 h. Expression of indicated proteins was determined by western blot. *, $p<0.05$ *versus* lane 1or siCtrl; †, $p<0.05$ *versus* lane 2; ‡, $p<0.05$ *versus* lane 3. $n=3$.

Table S1. Sequences of primers for qPCR analysis.

Gene	Forward	Backward
Homo *ATF4*	ATGACCGAAATGAGCTTCCTG	GCTGGAGAACCCATGAGGT
Homo *ATF6*	TCCTCGGTCAGTGGACTCTTA	CTTGGGCTGAATTGAAGGTTTTG
Homo *Ngbr*	GGGCATCTCCTACATTAGCG	CCCAGAAGTTCTTGCTGTTG
Homo *Nogo-B*	AATAGGCTGGCACCAAACAC	CGTGACAAGAGATGGACGGT
Homo *XBP1s*	CCGCAGCAGGTGCAGG	ACATGACTGGGTCCAAGTTGT
Homo *IRE1α*	CACAGTGACGCTTCCTGAAAC	GCCATCATTAGGATCTGGGAGA
Homo *BIP*	TTGACTCCGACCTTCACCTTCC	TTTCACAGTGGCCAAGAGTC
Homo *CHOP*	GGAAACAGAGTGGTCATTCCC	CTGCTTGAGCCGTTCATTCTC
Homo *SKIP*	AGGGGCGAGACATCCCAAA	AGTCCTCGATCCGAAAGTTCA
Homo β-*actin*	CTGGAACGGTGAAGGTGACA	AAGGGACTTCCTGTAACAATGCA
Mus *Ngbr*	GAGGAAGCCCACAGATCTGGATGTA	TCTGATTTGCCAGGGAAGAAAGCC
Mus *Nogo-B*	TCGGGCTCAGTGGTTGTT	GAGACAGCAGCAGGAATAAGCT
Mus *Gk*	TGAACCTGAGGATTTGTCAGC	CCATGTGGAGTAACGGATTTCG
Mus *Dgat*	GGTGCCCTGACAGAGCAGAT	CAGTAAGGCCACAGCTGCTG
Mus *Srebf1*	TGACCCGGCTATTCCGTGA	CTGGGCTGAGCAATACAGTTC
Mus *Acc1*	GAAGTCAGAGCCACGGCACA	GGCAATCTCAGTTCAAGCCAGTC
Mus *Fasn*	CTGCGATGAAGAGCATGGTTT	CCATAGGCGATTTCTGGGAC
Mus *Atf4*	CCTGAACAGCGAAGTGTTGG	TGGAGAACCCATGAGGTTTCA
Mus *Atf6*	TCGCCTTTTAGTCCGGTTCTT	GGCTCCATAGGTCTGACTCC

Mus *Xbp1s*	CTGAGGTCCGCAGCAGGT	TGTCAGAGTCCATGGGAAGA
Mus *Skip*	CAGCACGGAGACAGGAACAC	AGGCCACATTCCACGTCAC
Mus *Pparα*	AGTTCGGGAACAAGACGTTG	CAGTGGGGAGAGAGGACAGA
Mus *Pgc1α*	CCCTGCCATTGTTAAGACC	TGCTGCTGTTCCTGTTTTC
Mus *Sirt1*	GACGGTATCTATGCTCGCCT	ACACAGAGACGGCTGGAACT
Mus *Bip*	CGCTGGGCATCATTGAAGTAA	GAGGTGGGCAAACCAAGACAT
Mus *Chop*	CCACCACACCTGAAAGCAGAA	GGTGCCCCCAATTTCATCT
Mus *Mfn1*	ATGGCAGAAACGGTATCTCCA	GCCCTCAGTAACAAACTCCAGT
Mus *Mfn2*	AGAACTGGACCCGGTTACCA	CACTTCGCTGATACCCCTGA
Mus *Opa1*	TGGAAAATGGTTCGAGAGTCAG	CATTCCGTCTCTAGGTTAAAGCG
Mus *Drp1*	GCAACTGGAGAGGAATGCTG	CACAATCTCGCTGTTCTCGG
Mus *Fis1*	AGAGGAACAGCGGGACTATG	CCATGCCTACCAGTCCATCT
Mus *Mff*	CACCACCAAATGCTGACCTG	GGTGTTTTCAGTGCCAGAGG
Mus *Pink1*	CTGTCAGGAGATCCAGGCAATT	GTGGGCATGGTGGCTTCAT
Mus *Parkin*	CGTGTGATTTTTGCCGGGAAG	GGTCCACTCGTGTCAAGCTC
Mus *TNFα*	CGTCGTAGCAAACCACCAAG	TTGAAGAGAACCTGGGAGTAGACA
Mus *IL1β*	GACCTTCCAGGATGAGGACA	AGCTCATATGGGTCCGACAG
Mus *IL4*	ACAGGAGAAGGGACGCCAT	GAAGCCCTACAGACGAGCTCA
Mus *IL6*	GAGGATACCACTCCCAACAGACC	AAGTGCATCATCGTTGTTCATACA
Mus *IL10*	GCTCTTACTGACTGGCATGAG	CGCAGCTCTAGGAGCATGTG
Mus *β-actin*	ATGGAGGGGAATACAGCCC	TTCTTTGCAGCTCCTTCGTT

Homo, homo sapiens; Mus, mouse sapiens.

Primary antibodies

Mouse anti-protein kinase B (AKT, Cat# 5239S) monoclonal antibody, rabbit anti-phosphorylated-AKT (p-AKT, Cat# 4060L), phosphorylated glycogen synthase kinase 3 beta (p-GSK3β, Cat# 5558S), pancreatic endoplasmic reticulum kinase (PERK, Cat# 3192S), eukaryotic translation initiation factor 2α (EIF2α, Cat# 9722S), phosphorylated-eIF2α (p-EIF2α, Cat# 3597S), phosphorylated-ACC (Cat# 11818), ACC (Cat# 3676S), phosphorylated AMPKα (p-AMPKα, Cat# 2535S), insulin (Cat# 3014) monoclonal antibodies, and rabbit anti-phosphorylated-mTOR (p-mTOR, Cat# 2971), phosphorylated-IRS1 (p-IRS1, Cat# 3070S) polyclonal antibodies were purchased from Cell Signaling Technology (Danvers, MA, USA). Rabbit anti-GSK3β (Cat# 22104-1-AP), heat shock protein 90 (Hsp90) (Cat# 13171-1-AP), IRS1 (Cat# 17509-1-AR) and insulin (Cat# 15848-1-AP) polyclonal antibodies, mouse HRP-glyceraldehyde-3-phosphate dehydrogenase (GAPDH, Cat# HRP-60004) and mouse anti-mTOR (Cat# 66888-1-IG) monoclonal antibodies, HRP-conjugated goat anti-rabbit IgG (H+L, Cat# A00001-2), anti-mouse IgG (H+L, Cat# 00001-1) and goat anti-rabbit IgG (H+L)-Rhodamine (Cat# SA00007-2) antibodies were purchased from Proteintech Group (Chicago, IL, USA). Rabbit anti-AMPKα1 (Cat# NB110-55457) monoclonal antibody and rabbit anti-Nogo-B (Cat# NB100-56681) polyclonal antibody were purchased from Novus Biologicals (Littleton, CO, USA). Rabbit anti-NGBR (Cat# ab168351) monoclonal antibody and rabbit anti-Nogo-B (Cat# ab47085) polyclonal antibody were purchased from Abcam (Cambridge, MA, USA). Rabbit anti-β-actin (Cat# sc-130656) polyclonal antibody and mouse anti-glucagon (Cat# sc-514592) monoclonal antibody were purchased from Santa Cruz Biotechnology (Dallas,

Texas, USA). Rabbit anti-binding immunoglobulin protein or 78 kDa glucose-regulated protein (BIP/GRP78, Cat# A11366), phosphorylated-Raptor (p-Raptor, Cat# AP0928), Raptor (Cat# A8992) polyclonal antibodies were purchased from ABclonal (Wuhan, China). Goat anti-mouse IgG (whole molecule)-FITC (Cat# F0257) antibody was purchased from Sigma-Aldrich.