SUPPLEMENTARY DATA

Materials and Methods

Animal studies

All animals were maintained and used in accordance with the guidelines of the Institutional Animal Care and Use Committee of the Institute for Nutritional Sciences. All of the experimental procedures were carried out in accordance with the CAS ethics commission with an approval number 2010-AN-8. All mice were housed at a temperature of 25^{o}C under a 12-hr dark/light cycle. C57BL/6J mice were obtained from Shanghai Laboratory Animal Co., Ltd. (SLAC, Shanghai, China). *Paqr3*-null mice were generated as previously described with deletion of the exon 2 whole (1; 2). The *Paqr3*$^{-/-}$ mice were crossed with C57BL/6J for at least 5 generations. For studies of *in vivo* insulin signaling, male mice fed with normal chow at 8 to 10 weeks of age were fasted over night, followed by i.p. injection of insulin (5 units/kg body weight). The mice were sacrificed after 5 minutes and the liver and gastrocnemius muscle were excised and snap-frozen for immunoblotting analyses. For high fat diet (HFD) feeding, 8-week-old wild-type mice were fed with HFD (Research Diets, Inc., NJ, USA) for 16 weeks.

Plasmids

The full-length human p110α and p85α plasmids were purchased from Addgene (Cambridge, MA, USA). P110α was subcloned into p3Xflag-CMV-10 vector (Sigma-Aldrich, St. Louis, MO, USA) and p85α was subcloned into the mammalian expression vector pcDNA3.1 (Invitrogen, Carlsbad, CA, USA) with a HA epitope tag added to the N-terminus by a PCR-based method. The Myc-tagged PAQR3 and GFP-tagged PAQR3 were described previously (1). The PAQR3 short hairpin RNA (shRNA) construct was used as previously reported (3). The mouse PH domain of AKT2 was isolated from mouse liver by reverse transcription PCR, confirmed by DNA sequencing, and cloned into pEGFP-C1 vector. The human p110αtruncation mutants (with Myc tags at the N-terminus) were generated by a PCR-based method and comprised of the p85 binding domain (p85BD, corresponding to aa 31-108), the Ras binding domain (RBD, corresponding to aa 173-292), the C2 domain (corresponding to aa 325-484), the helical domain (HD, corresponding to aa 525-696), and the catalytic domain (CD, corresponding to aa 699-1064).

Antibodies, co-immunoprecipitation and immunoblotting

The antibodies were purchased as follows: phosphor-IRβ(Tyr-1150/1151), total IRβphospho-AKT(Ser-473), total AKT, and phospho-GSK3β(Ser-9) from Cell Signaling Technology (Danvers, MA, USA); antibodies against phospho-IRS-1(Tyr-608) and phospho-tyrosine from Millipore (Upstate, MA, USA); antibodies against Myc, HA, and β-actin from Santa Cruz Biotechnology (Santa Cruz, CA, USA); antibodies against FLAG tag and Golgin-97from Sigma–Aldrich (St. Louis, MO, USA); antibodies against GM130 and p110α from Abcam (Cambridge, MA, USA); Alexa fluor 488 donkey anti-mouse IgG, Alexa Fluor 546 goat anti-mouse, rabbit IgG and Hoechst 33342 from Molecular Probes (Eugene, OR, USA); Cy-5-labeled goat anti-mouse, rabbit IgG from Jackson ImmunoResearch (Baltimore Pike, PA, USA). The co-immunoprecipitation assay and immunoblotting were performed as previously described (1; 4).

Cell culture

Mouse hepatocytes were isolated from livers by a modified two-step collagenase perfusion protocol (5). The hepatocytes were plated in collagen I coated 6-well plates (2×10^5 cells/well) in DMEM with 25 mM glucose (GIBCO, Invitrogen, Carlsbad, CA, USA) supplemented with 10% FBS (Invitrogen) and 1% penicillin/streptomycin. For insulin treatment, the cells were maintained in DMEM for 12 hours, followed by incubation with 10 nM of insulin (Sigma-Aldrich, St. Louis, MO, USA). For glucosamine

treated insulin resistance, primary hepatocytes were incubated for 18 hours in DMEM with 18 mM glucosamine. HEK293T, HepG2 and HeLa cells were maintained in DMEM supplemented with 10% FBS and 1% penicillin/streptomycin. Cell transfection was performed as previously described (4). For insulin treatment, HepG2 cells were maintained in DMEM for 18 hours, and then incubated with insulin (100 nM) for different length of time as indicated. Confocal analysis was performed as previously described (1; 4). For glucosamine treated insulin resistance, primary hepatocytes were incubated for 18 hours in DMEM with 18 mM glucosamine.

RNA isolation and real-time polymerase chain reaction (RT-PCR)

The cells or frozen tissues were lysed in TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Total RNA was purified and reverse-transcribed according to the manufacturer's instruction (TaKaRa, Shanghai, China). Real-time quantitative PCR was conducted with ABI Prism 7900 sequence detection system following the manufacturer's recommendations (Applied Biosystems, Foster City, CA, USA). The primers are as follows: 5'- GATGGCATTGGATTATGCAG-3' and 5'-AAGCACGGTGATCAGGTACA -3' for PAQR3, 5'-GATCATTGCTCCTCCTGAGC-3' and 5'-ACTCCTGCTTGCTGATCCAC-3' for actin.

ITT and GTT

The insulin tolerance test (ITT) and glucose tolerance test (GTT) were performed as previously described (6). In brief, for ITT, the mice were fasted for 4 hours; glucose concentrations were measured in blood collected from tail vein, immediately before and 30, 60 and 120 min after i.p. injection with human insulin (Novo Nordisk, Bagsvaerd, Denmark) at 0.75 units/kg body weight. For GTT, the mice were fasted overnight, glucose concentrations were measured at the indicated time after i.p. injection of glucose (1 g/kg body weight).

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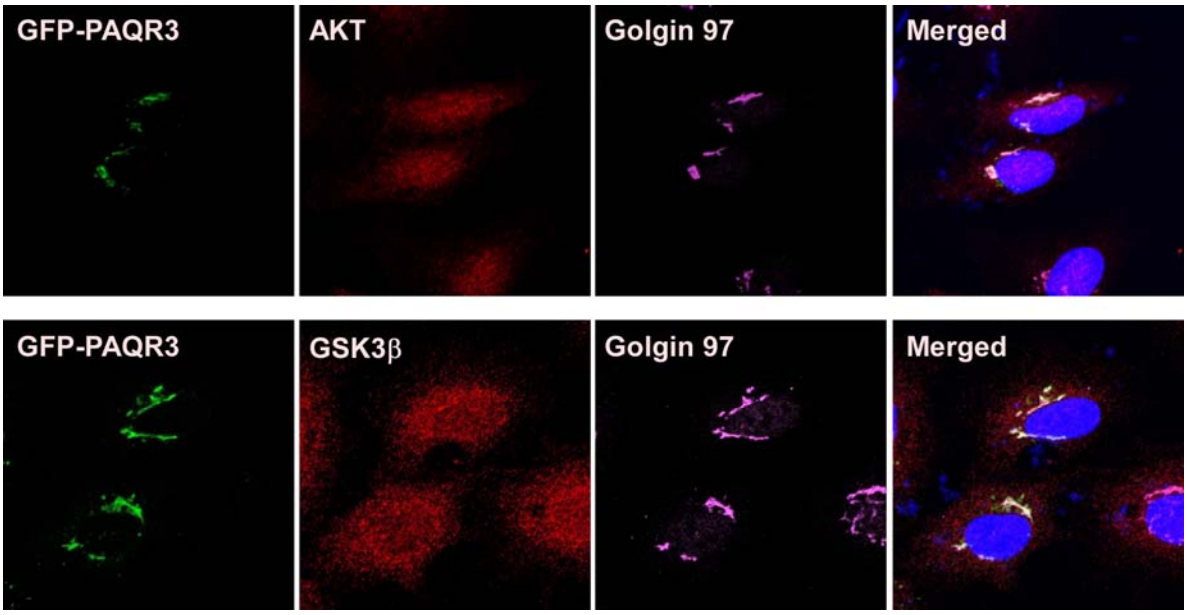
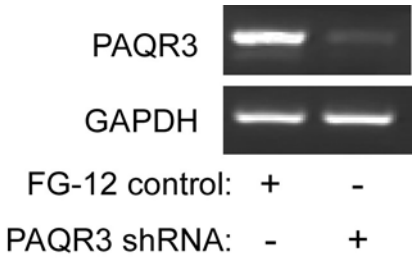
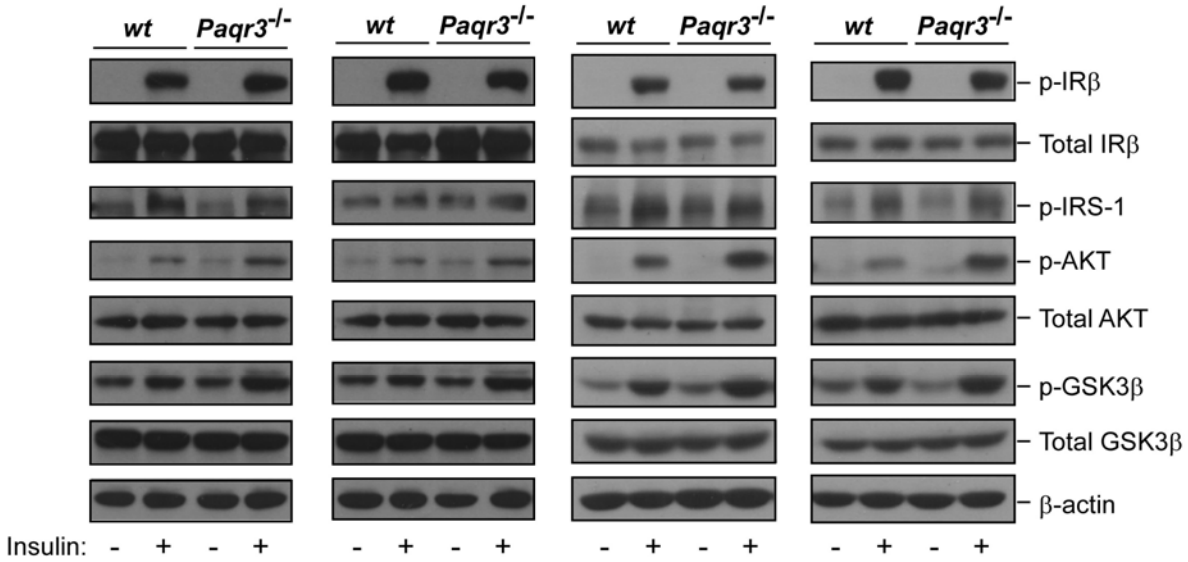
Supplementary Figure 1. Overexpression of PAQR3 cannot tether AKT and GSK3βto the Golgi apparatus. HeLa cells were transiently transfected with GFP-fused PAQR3, followed by immunofluorescence staining to detect endogenous AKT and GSK3β. The Golgi apparatus was stained with an anti-Golgin-97 antibody. The nuclei were stained with Hoechest 33342.



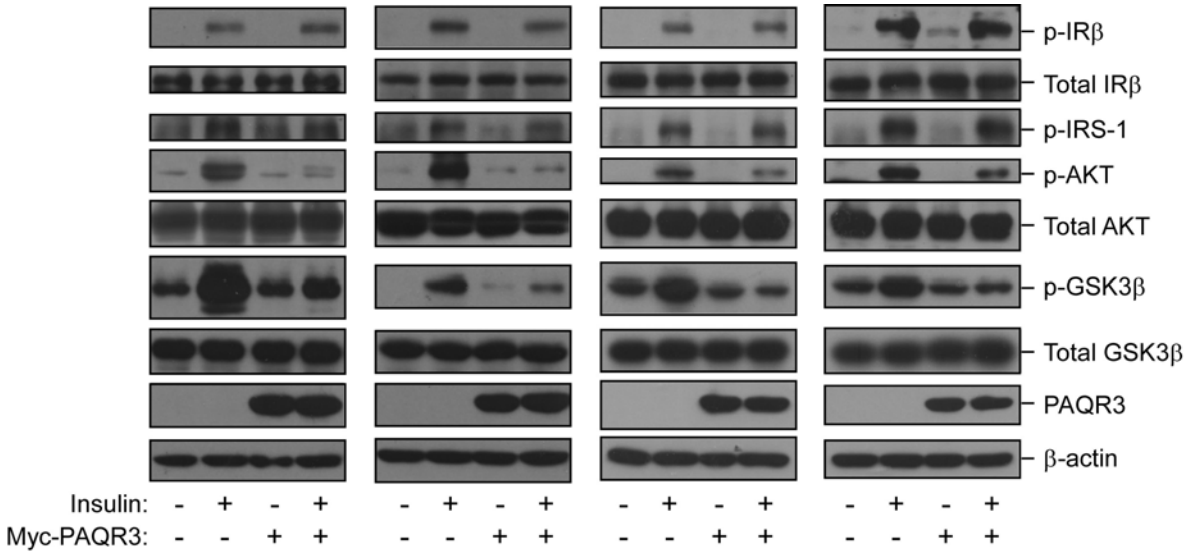
Supplementary Figure 2. Characterization of PAQR3 shRNA. HEK293T cells were transiently transfected with FG-12 control vector or PAQR3 shRNA plasmid. The mRNA level of PAQR3 at 48 hours after transfection was detected by RT-PCR.



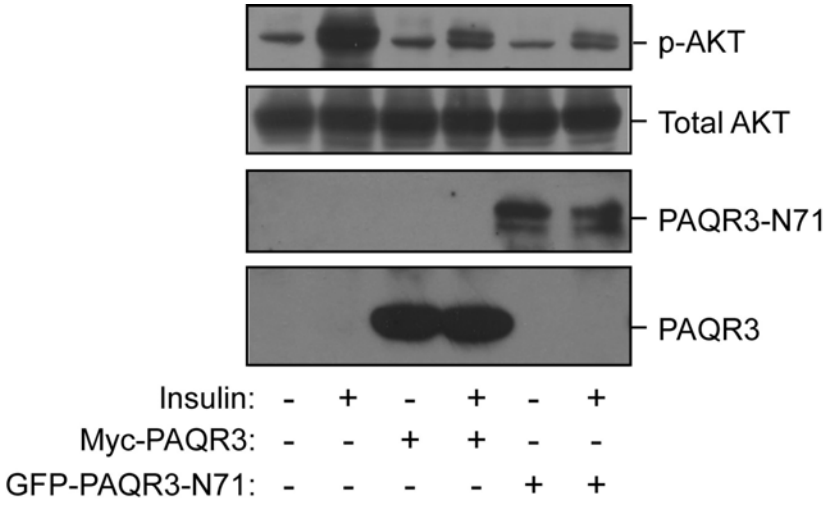
Supplementary Figure 3. Insulin signaling is enhanced by *Paqr3* deletion in hepatocytes Primary hepatocytes were isolated from *Paqr3*-deleted mice and their littermate controls. The cells were treated with insulin (10 nM) for 15 minutes, followed by immunoblotting with antibody as indicated. Five independent experiments were performed and gave rise to similar results. One of the results is shown in Figure 5A and the other four results are shown here.



Supplementary Figure 4. Overexpression of PAQR3 inhibits insulin signaling HepG2 cells were transiently transfected with a Myc-tagged PAQR3 plasmid as indicated, treated with insulin (100 nM) for 15 min, followed by immunoblotting. The expression of transfected PAQR3 was detected by an anti-Myc antibody. Five independent experiments were performed and gave rise to similar results. One of the results is shown in Figure 5B and the other four results are shown here.



Supplementary Figure 5. PAQR3-N71 is able to inhibit insulin-stimulated AKT phosphorylation HepG2 cells were transiently transfected with a Myc-tagged PAQR3 and GFP-PAQR3-N71 plasmids as indicated, treated with insulin (100 nM) for 15 min, followed by immunoblotting with the antibodies as indicated. The expression of transfected PAQR3 and PAQR3-N71 was detected by an anti-Myc and anti-GFP antibodies respectively. Please note that the first four lanes in the immunoblot are identical to the pictures shown in the left panel of Supplementary Figure 4.



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