Supporting Information

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SI Materials and Methods

Plasmids and Antibodies. Construction of a majority of the phosphatase and tensin homolog deleted on chromosome 10 (PTEN) and phosphatidylinositol 3,4,5-trisphosphate-dependent Rac exchanger 2 (P-REX2) plasmids used have been reported previously (1). Bacterial expression plasmids encoding GST-PTEN domains were made by PCR of the full-length GST-PTEN plasmid and subcloning into the PGEX-2TK vector. GST-PTEN-3E and FLAG-PTEN-3E mutants were made by amino acid substitution using site-directed mutagenesis (Agilent). FLAG-PTEN deletion mutants were constructed by substitution of a stop codon at the desired amino acid by site-directed mutagenesis. PTEN antibodies were purchased from Cell Signaling Technology or Santa Cruz Biotechnology. Monoclonal M2 FLAG and actin antibodies were purchased from Sigma and a monoclonal V5 antibody was from Invitrogen. V5 resin was obtained from Sigma. Antibodies against phospho-AKT (Thr308), phospho-AKT (Ser473), total AKT, phospho–GSK3-β (Ser9), phospho-FOXO1/3a (Thr24/Thr32), phospho-EGFR (Tyr1173), phospho-PDGFR (Tyr754), and phospho-IGF1Rβ (Tyr1135/1136)/IRβ (Tyr1150/1151) were purchased from Cell Signaling Technology. A P-REX2 rabbit polyclonal antibody to amino acids 960–973 was made by Zymed Laboratories. Phosphotyrosine 4G10 was obtained from Millipore. Secondary antibodies directed against rabbit and mouse IgG conjugated to HRP were purchased from Pierce.

Cell Culture and Transfections. HEK293 cells were grown in DMEM supplemented with 10% (vol/vol) FBS plus 100 IU penicillin and 100 μg/mL streptomycin (Cellgro). U87 cells were grown in MEM Eagle (Cellgro) supplemented with 10% (vol/vol) FBS and 100 IU penicillin and 100 μg/mL streptomycin. For signaling experiments, cells were starved in medium without FBS for 16 h before lysing. Transfections were performed using Lipofectamine 2000 (Invitrogen) following the protocol of the manufacturer.

Immunoblotting. Samples lysed in 2× Laemlli sample buffer [125 mM Tris pH 6.8, 10% (vol/vol) 2-mercaptoethanol, 4% (wt/vol) SDS, 20% (vol/vol) glycerol, 0.05% bromophenol blue] were boiled for 5 min. Samples were separated by SDS/PAGE on Tris-glycine gels (Invitrogen) and transferred onto PVDF using a semidry apparatus (Millipore). Membranes were blocked with 5% (wt/vol) nonfat milk in Tris-buffered saline and Tween 20 buffer (TBST) and incubated with the appropriate antibody overnight at 4 °C. Membranes were washed three times with TBST and the appropriate secondary antibody was added for 1 h at room temperature. Blots were developed using ECL (Pierce) and audioradiography film (Denville).

GST Fusion Proteins. Recombinant GST fusion proteins were transformed into BL21(DE3) pLyseE bacterial cells (Invitrogen) and protein was purified as described previously (1). Briefly, expression of protein was induced in bacterial cultures growing at log phase with 0.1 mM Isopropyl β-D-1-thiogalactopyranoside (Sigma) at room temperature for 16 h. Protein was extracted by sonication in lysis buffer (400 mM NaCl, 50 mM Tris pH 7.2, 1% Triton X-100, 1 mM EDTA) and incubated with glutathione Sepharose beads (Sigma) overnight and washed excessively with either BC200 buffer [25 mm Tris·HCl buffer, pH 7.4, 200 mm KCl, 0.2% Triton X-100, 1 mm EDTA, and 10% (vol/vol) glycerol] or phosphatase buffer (150 mM NaCl, 25 mM Tris·HCl pH 7.5) (PB) if it was to be eluted. To elute, beads were incubated with an equal volume of glutathione elution buffer (150 mM NaCl, 25 mM Tris·HCl, 50 mM glutathione, pH 8.0) for 30 min at room temperature. Purified proteins were dialyzed in 5 L of elution buffer without glutathione overnight at 4 °C using SnakeSkin dialysis tubing (Pierce). Proteins conjugated to beads and purified proteins were run on a gel with BSA standards and quantified by Coomassie staining.

In Vitro Transcription and Translation. V5-tagged P-REX2 domains were in vitro transcribed and translated using the TnT T7 Quick Coupled Rabbit Reticulocyte Lysate kit (Promega) per the manufacturer's protocol.

GST Pull-Down Assay. Rabbit reticulocyte lysates (10 μL) were incubated with purified GST Sepharose or GST PTEN Sepharose in BC200 [25 mm Tris·HCl buffer, pH 7.4, 200 mm KCl, 0.2% Triton X-100, 1 mm EDTA, and 10% (vol/vol) glycerol] at 4 °C for 4 h. The Sepharose was then washed extensively with BC200 and eluted with sample buffer.

Protein Purification. V5-tagged P-REX2 domains were purified from HEK293 cells as previously described with a few modifications (1). Briefly, HEK293 cells growing in 15-cm dishes were transfected with pCDNA3.1/V5/His plasmids and incubated with MG132 16 h before lysing. At 36 h after transfection, cells were washed with cold TBS and lysed with high salt lysis buffer (500 mM NaCl, 25 mM Tris pH 7.4, 0.1% Triton X-100, 1 mM EDTA) plus eukaryotic protease inhibitor mixture. Clarified lysate was precleared with mouse IgG and protein A/G PLUS agarose for 1 h at 4 °C and protein was then immunoprecipitated using V5 agarose overnight at 4 °C. Beads were washed on a poly-prep column (BioRad) once with lysis buffer and five times with PB. Protein was eluted with V5 peptide in PB at a concentration of 150 μg/mL. Protein was quantified with spectrophotometry A280 and visualized by Western blot.

Immunoprecipitation. Transfected cells were rinsed with cold PBS and lysed with Triton-containing lysis buffer (150 mM NaCl, 25 mM Tris pH 7.4, 0.1% Triton X-100, 1 mM EDTA) supplemented with eukaryotic protease mixture inhibitor (Sigma) 36 h after transfection. Lysates were vortexed, sonicated, and clarified by high-speed centrifugation for 30 min, and precleared with normal mouse IgG with protein A/G PLUS agarose (Santa Cruz Biotechnology) for 1 h at 4 °C. Protein complexes were then immunoprecipitated using either V5 agarose or FLAG antibody (Sigma) with 20 μL protein A/G PLUS agarose overnight at 4 °C. Beads were washed extensively with lysis buffer and eluted in 2× Laemmli buffer.

PTEN Phosphatase Assay. For in vitro phosphatase assays, equimolar concentrations of purified V5-tagged P-REX2 protein and recombinant GST fusion protein were incubated in phosphatase buffer (150 mM NaCl, 25 mM Tris·HCl pH 7.5) at a total volume of 48 μL. The protein mixture was preincubated at 37 °C for 10 min before 20 μM soluble di-C8-D-myo-phosphatidylinositol 3,4,5-trisphosphate (PIP3) (Echelon) was added. The reactions were incubated at 37 °C for 30 min and stopped with 100 μL Biomol green reagent (Enzo). A reaction mixture containing buffer and PIP3 only was run as a control. The colorimetric reaction developed for 15 min before being read on a plate reader at an absorbance of 620 nm, and background values from the control reaction were subtracted out. For immunoprecipitation phosphatase assays, 10-cm plates of HEK293 or U87 cells were

cotransfected in triplicate with either 5 μg of V5- or FLAG-tagged PTEN plasmid and 15 μg of control or V5-tagged P-REX2 constructs using Lipofectamine 2000 (Invitrogen). After 36 h, cells were lysed with Triton-containing lysis buffer, vortexed, sonicated, and centrifuged at high speed for 30 min. Clarified lysates were precleared with normal mouse or rabbit IgG and protein A/G PLUS agarose for 1 h at 4 °C. A total of 1 mg of total protein was next incubated with either V5 agarose or PTEN 138G6 antibodies (Cell Signaling Technology) overnight at 4 °C, and the beads were then washed twice with lysis buffer and four times with PB. The beads were preincubated in PB at 37 °C for 10 min and then collected by centrifugation at low speed to remove the supernatant. Beads were then incubated with 20 μM PIP3 and PB at a final volume of 50 μL and incubated at 37 °C for 30 min. The beads were removed from the reaction mixture by centrifugation at low speed, and the supernatant was added to 100 μL Biomol green reagent, which stopped the reaction, and absorbance was read at 620 nm after 15 min. Phosphate standards were used to quantitate free phosphate released, and background values from control reactions were subtracted out.

Generation of *Prex2* Knockout Mice and Genotyping. To generate mice deleted for *Prex2*, the Sanger Institute Gene Trap Resource was used. In brief, the E14 embryonic stem cell (ES) line clone AH0440 was purchased from the Wellcome Trust Sanger Institute. In brief, this cell line was created by high-throughput gene trapping, resulting in the insertion of a reporter gene containing a β-galactosidase and neomycin phosphotransferase II fusion between exons 4 and 5 of *Prex2*. The location of this reporter gene in the ES cells was confirmed by RT-PCR. The mutant allele was transmitted through the germ line, and mice heterozygous for the mutant allele were intercrossed to generate homozygous mutant progeny. Mutant mice were then backcrossed with C57BL/6 mice for eight generations to generate mice of the same background. Genomic DNA for genotyping was isolated from tail samples from 3-wk-old mice. Genotyping the mutant allele was done by PCR using forward primer 5′ TGA TAG GAT GCATGG GAC AA 3′, which anneals to intron 4 outside of the reporter gene, and reverse primer 5′ CAA CCT CCG CAA ACT CCT AT 3′, which anneals to the reporter gene. Genotyping the wild-type allele was done using forward primer 5′ TCG ACT CCT GAA GAT TTG ACC 3′ and reverse primer 5′ TGA CCA CGT TGC CTT GACTA 3′, which amplified a region of intron 4 that is deleted in the mutant allele. All mouse studies were approved by the Institutional Animal Use Committee at Icahn School of Medicine at Mount Sinai.

Harvest and Culture of Mouse Embryonic Fibroblasts. Embryos were harvested between days 12.5–14.5. The head, which was used for genotyping, liver, and large blood clots were removed, and the embryo was washed in 2 mL of PBS. The tissue was minced with a razor blade, and 2 mL of 0.05% tryspin-EDTA (Cellgro) was added. Minced tissue was incubated at 37 °C for 10 min and then pipetted vigorously until cells were in a single cell suspension. A total of 1 mL of cells was placed in a T25 flask. Mouse embryonic fibroblasts (MEFs) were cultured in DMEM supplemented with 10% (vol/vol) FBS (Invitrogen), 1% of 200 mM L-glutamine, plus 100 IU penicillin and 100 μg/mL streptomycin (Cellgro). For growth factor stimulation, MEFs were starved in DMEM for 3 h and then incubated with 10 μg/mL bovine insulin, 20 ng/mL IGF1, 20 ng/mL PDGF, and 20 ng/mL EGF as indicated (Sigma).

Cell Proliferation Assay. A total of 4,000 cells were plated per well in a 48-well plate in at least triplicate for each experiment. Cells were then allowed to proliferate and were fixed at indicated time points in 0.05% crystal violet in 10% (vol/vol) formalin. Each well was then washed multiple times with PBS. For relative quantification of cell density, the crystal violet was resolubilized in 10% (vol/vol) acetic acid and the absorbance at 595 nm was recorded by a MicroQuant plate spectrophotometer.

In Vivo Insulin Signaling. Eight-week-old male mice were fasted overnight. For collection of starved tissue, fasted mice were killed by cervical dislocation and liver, fat, and hind leg skeletal muscle was collected from the starved samples. For insulin stimulation, fasted mice were injected with 0.75 mU/g bovine insulin intraperitoneally and liver, fat, and skeletal muscle was collected 2 min or 15 min after stimulation. Tissues were flash frozen and stored at −80 °C. For Western blot analysis, frozen tissue was thawed on ice and homogenized for 30 s at high speed using a Tissuemiser (Fisher Scientific) in Triton-containing lysis buffer supplemented with eukaryotic protease mixture inhibitor.

PTEN Phosphatase Assays from Cells and Tissue. *Prex2*$^{+/+}$, *Prex2*$^{-/-}$, and *Pten*$^{-/-}$ (negative control) (2) MEFs were starved in plain DMEM for 3 h and then stimulated with 10 μg/mL insulin for 10 min. Cells were washed with cold TBS and lysed with Triton-containing lysis buffer supplemented with eukaryotic protease mixture inhibitor. Flash-frozen liver tissue from mice fasted for 16 h or stimulated with bovine insulin at 10 mU/g for 8 min, and flash-frozen human adipose tissue was homogenized in Triton-containing lysis buffer for 30 s. Lysates were then clarified by high-speed centrifugation for 30 min and precleared with normal rabbit IgG and protein A/G PLUS agarose for 1 h at 4 °C. One milligram of total protein was next incubated with PTEN 138G6 antibody or rabbit IgG (negative control for tissue samples) conjugated to agarose beads overnight at 4 °C, and the beads were then washed extensively with PB. The beads were preincubated in PB at 37 °C for 10 min and then collected by centrifugation at low speed to remove the supernatant. Beads were then incubated with 20 μM soluble di-C8-D-myo-PIP3 and PB at a final volume of 50 μL and incubated at 37 °C for 30 min. The beads were removed from the reaction mixture by centrifugation at low speed, and the supernatant was added to 100 μL Biomol Green reagent, which stopped the reaction, and absorbance at 620 nm was read after 15 min. Phosphate standards were used to quantitate free phosphate released, and background values from control reactions were subtracted out.

Fibroblast Endogenous Coimmunoprecipitation. MEFs were immortalized with dominant-negative p53, and 1×10^7 cells were starved or stimulated with 10 μg/mL insulin for 10 min. Cells were then washed with PBS and lysated in lysis buffer (150 mM NaCL, 50 mM Hepes, 1 mM EDTA, 1% Nonidet P-40, 0.025% deoxycholate, 1 mM NaF, 1 mM NaVO4, protease inhibitor mixture). Lysates were clarified by high-speed centrifugation for 30 min and precleared with normal goat IgG with protein A/G PLUS agarose (Santa Cruz Biotechnology) for 1 h at 4 °C. Protein complexes were then immunoprecipitated using PTEN N-19 antibody (Santa Cruz Biotechnology) with 40 μL protein A/G PLUS agarose overnight at 4 °C. Beads were washed extensively with lysis buffer and eluted in 2× Laemmli buffer. Pten and P-rex2 protein present in the eluted samples and total cell lysate were visualized by SDS/PAGE on 4–12% Tris-glycine gels.

Liver Endogenous Coimmunoprecipitation. Fresh mice livers were homogenized in lysis buffer. Lysates were clarified by high-speed centrifugation for 30 min and precleared with normal goat IgG with protein A/G PLUS agarose (Santa Cruz Biotechnology) for 1 h at 4 °C. Protein complexes were then immunoprecipitated using PTEN N-19 antibody (Santa Cruz Biotechnology) with 40 μL protein A/G PLUS agarose overnight at 4 °C. Beads were washed extensively with lysis buffer and eluted in 2× Laemmli buffer. Pten and P-rex2 protein present in the eluted samples and total cell lysate were then visualized by SDS/PAGE on 4–12% Tris-glycine gels.

In Vitro PI3K Activity Assay. Fresh liver samples from fasted mice or mice stimulated with bovine insulin at 10 mU/g for 8 min. were homogenized in PI3K buffer (25 mM Tris, pH 7.5, 10 mM EDTA, 10 mM EGTA, 50 mM $Na_4P_2O_7$, 1% Nonidet P-40, 10 mM Na_3VO_4, 25 mM NaF, protease inhibitor mixture). Lysates were incubated with anti–phosphor-Tyr-4G10 antibody for 1 h, washed twice with PI3K buffer, and three times with TNE [10 mM Tris (pH 7.5), 100 mM NaCl, 1 mM EDTA]. The reaction was set up by adding 4 μg phosphoinositide, 12 μg phosphoserine (Avanti Polarlipids), 5.5 μL Hepes (100 mM, pH = 7.0), 2.0 μL $MgCl_2$, 0.5 μL ATP (10 mM), and 10 μCi (γ-32P) ATP (PerkinElmer) to 70 μL of immunoprecipitate. Reactions were carried out at room temperature and stopped with 25 μL of 5 M HCl after 10 min. The lipids were extracted with 160 μL of CHCl3:MeOH (1:1), spotted on TLC silica gel plates (Thomas Scientific) and separated overnight with 1-propanol:2 M acetic acid (65:35). The radioactivity was visualized and quantified using a Trio Typhoon PhosphorImager (GE Healthcare).

Mass Spectrometry Measurements of Inositol Lipids. Mass spectrometry was used to measure inositol lipid levels essentially as described previously (3), using a QTRAP 4000 (AB Sciex) mass spectrometer and using the lipid extraction method described for mouse liver tissue. Measured C18:0 C20:4 PIP3 signals from each sample were expressed as a ratio of the C18:0 C20:4 PIP2 signal to account for cell variation in each sample.

Glucose Challenge and Insulin Tolerance Test. For glucose challenge tests, 6-wk-old male mice were starved overnight. After measuring fasting blood glucose levels using the OneTouch Ultra glucometer, mice were injected intraperitoneally with 2 mg dextrose/g body weight. Blood glucose was measured at 15, 30, 45, 60, 90, and 120 min time points. For insulin tolerance tests, 8-wk-old fed male mice were injected intraperitoneally with 0.75 units/kg bovine insulin (Sigma), and blood glucose was measured before injection and postinjection at 15, 30, 45, 60, 90, and 120 min.

Human Studies. All subjects were recruited from either the University of Arkansas for Medical Sciences or the University of Kentucky and signed consent forms were approved by the respective Institutional Review Boards. All subjects were healthy, except for obesity in some, and none were diabetic or taking any medications likely to affect adipocyte metabolism. These subjects were recruited for studies on obesity and insulin resistance and have been included in previous studies (4). After an overnight fast, an incisional adipose biopsy was performed from the abdominal s.c. wall under local anesthetic and immediately frozen. Insulin sensitivity was measured using the frequently sampled i.v. glucose tolerance test with minimal model analysis, as described previously (5).

Statistical Analysis. *P* values were calculated by unpaired Student *t* tests.

1. Fine B, et al. (2009) Activation of the PI3K pathway in cancer through inhibition of PTEN by exchange factor P-REX2a. *Science* 325(5945):1261–1265.
2. Lesche R, et al. (2002) Cre/loxP-mediated inactivation of the murine Pten tumor suppressor gene. *Genesis* 32(2):148–149.
3. Clark J, et al. (2011) Quantification of PtdInsP3 molecular species in cells and tissues by mass spectrometry. *Nat Methods* 8(3):267–272.
4. Spencer M, et al. (2011) Adipose tissue extracellular matrix and vascular abnormalities in obesity and insulin resistance. *J Clin Endocrinol Metab* 96(12):E1990–E1998.
5. Bergman RN, Prager R, Volund A, Olefsky JM (1987) Equivalence of the insulin sensitivity index in man derived by the minimal model method and the euglycemic glucose clamp. *J Clin Invest* 79(3):790–800.

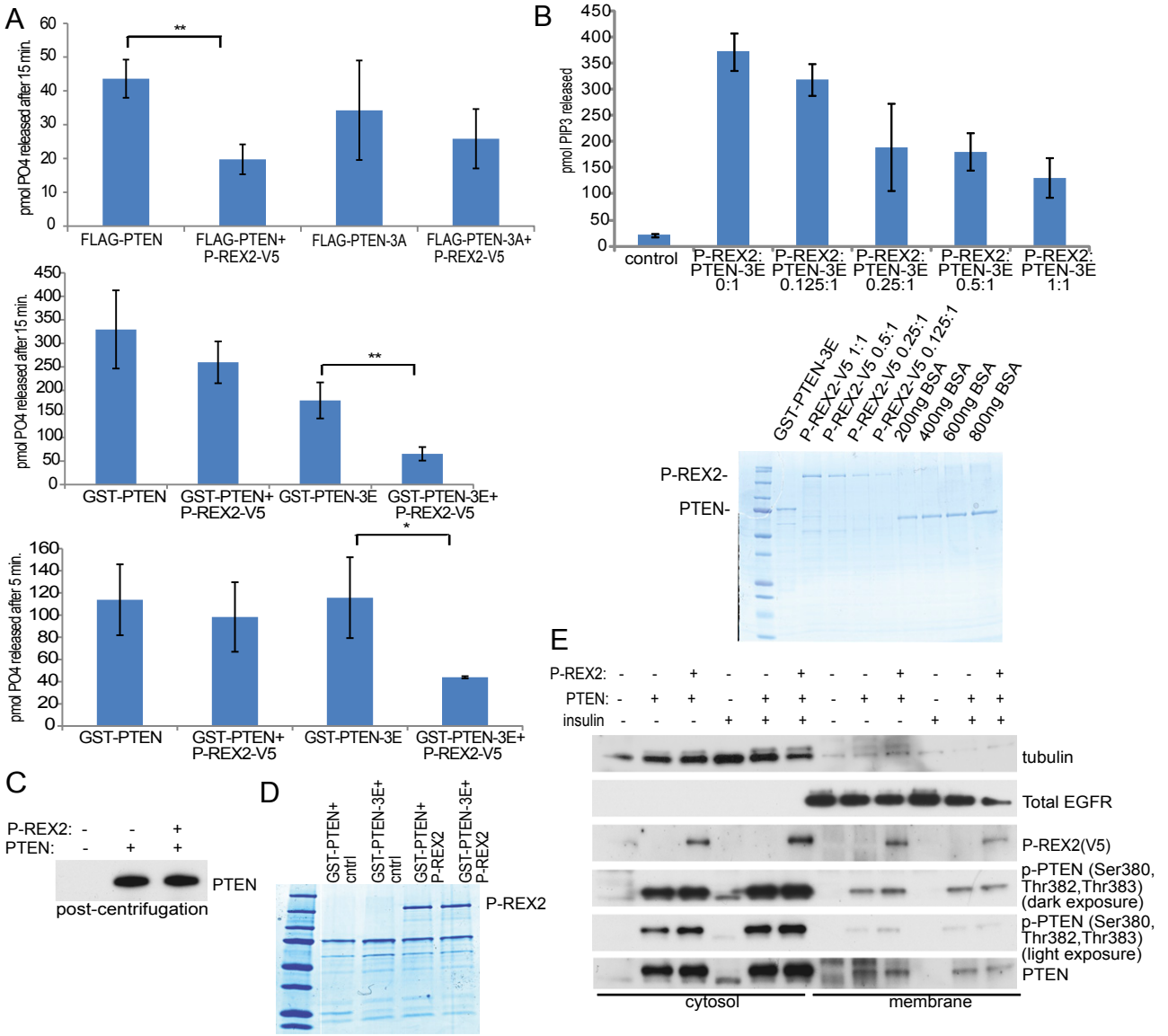

Fig. S1. Stoichiometric inhibition of PTEN by P-REX2 does not affect PTEN stability or phosphorylation. (*A*) To determine if GST-PTEN and FLAG-PTEN are similarly sensitive to P-REX2 inhibition at earlier time points, the phosphatase activity of PTEN was assayed with PIP3 at 37 °C for either 15 min or 5 min. $*P < 0.05$, $**P < 0.01$. (*B*) To determine the stoichiometry of P-REX2 inhibition of PTEN, purified P-REX2-V5 was incubated with GST-PTEN-3E at decreasing molar ratios. Phosphatase activity of PTEN was assayed with PIP3 at 37 °C for 30 min. Levels of purified protein used in each reaction are shown by Coomassie staining. (*C*) GST-PTEN-3E was incubated with equimolar amounts of purified P-REX2-V5 in the presence of PIP3 at 37 °C for 30 min. Reaction mixtures were spun down at high speed for 2 h at 4 °C, and levels of precipitated PTEN were determined by Western blot. (*D*) HEK293 cell lysates expressing empty vector or P-REX2-V5 were incubated with recombinant GST-PTEN or GST-PTEN-3E conjugated to Sepharose beads. Levels of pulled-down proteins were determined by Coomassie staining. (*E*) FLAG-PTEN was transfected into U87 cells alone or in combination with P-REX2-V5. Cytosolic and membrane fractions of starved or insulin-stimulated cells were isolated, and levels of C-terminal phosphorylated or total PTEN were determined by immunoblotting.

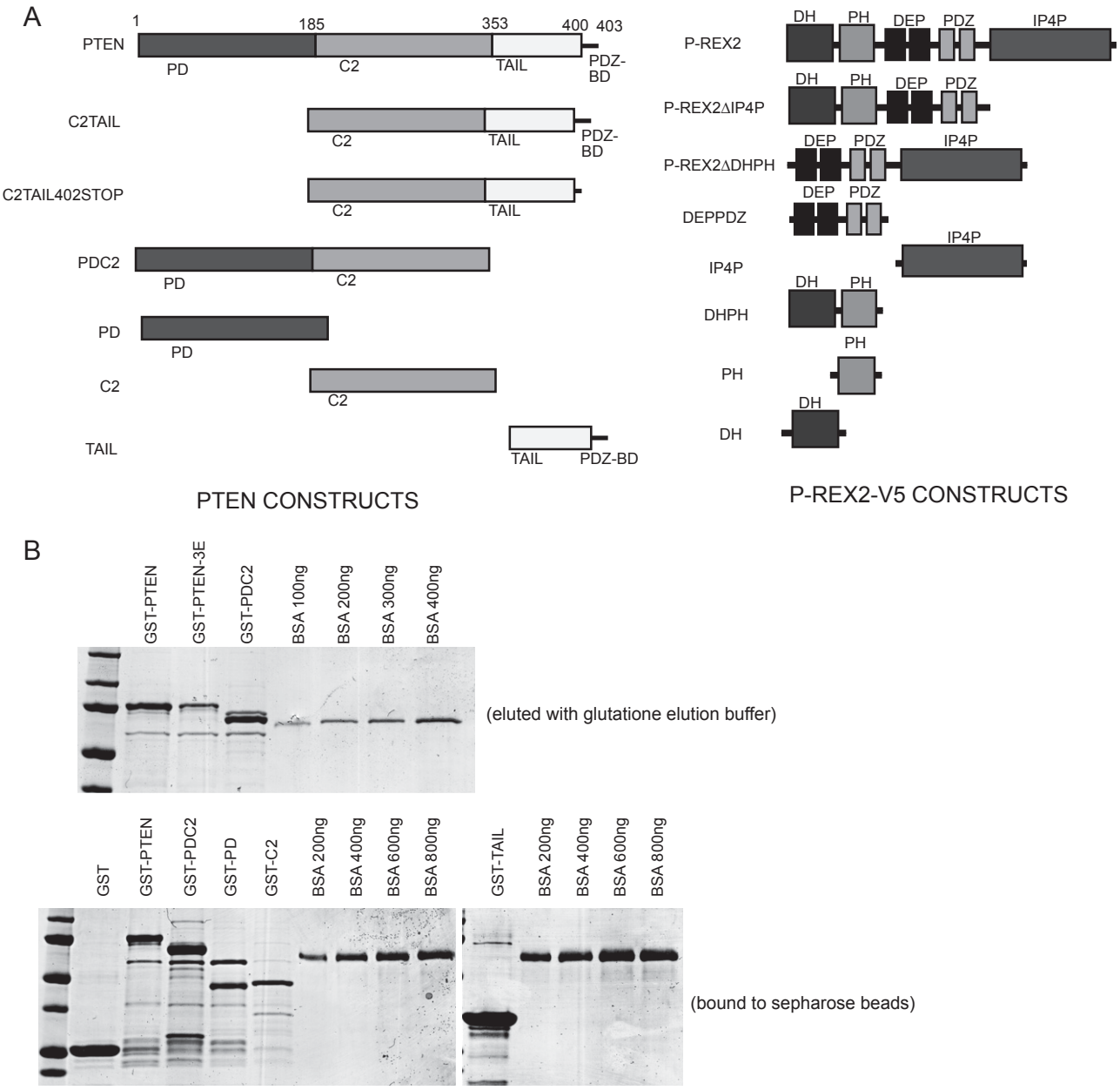

Fig. S2. Description and quantification of constructs used. (*A*) Representative illustration of deletion mutants used in experiments. (*B*) Coomassie stain of recombinant full-length GST-PTEN WT and GST-PTEN deletion constructs purified from BL21(DE3) pLyseE bacterial cells using glutathione Sepharose beads. BSA standards were used to quantify the protein.

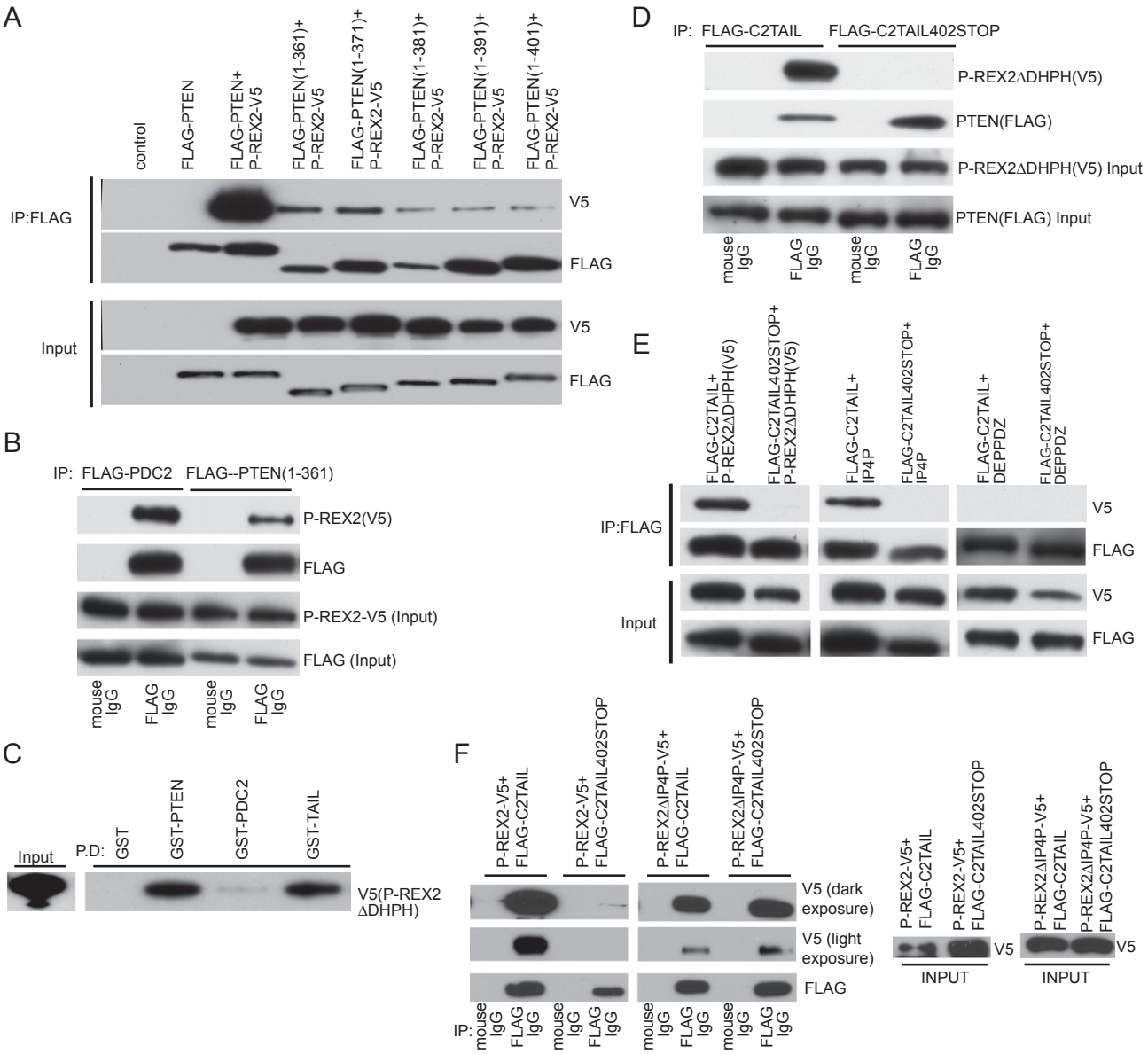

Fig. S3. P-REX2 interacts with the PDZ-binding domain of PTEN. (*A* and *B*) P-REX2-V5, FLAG-PTEN C-terminal tail deletion mutants, or empty vectors were cotransfected into HEK293 cells and lysates were incubated with FLAG-M2 antibody or normal mouse IgG conjugated to agarose beads. Total lysates and FLAG-immunoprecipitated proteins were analyzed by Western blot. (*C*) HEK293 cell lysate expressing P-REX2ΔDHPH-V5 was incubated with recombinant GST-PTEN, GST-PDC2, GST-TAIL, or control GST Sepharose beads. Pulled-down proteins were analyzed by immunoblotting. (*D*) HEK293 lysates coexpressing P-REX2ΔDHPH domains with FLAG-C2TAIL or FLAG-C2TAIL402STOP were incubated with FLAG-M2 antibody or normal mouse IgG conjugated to agarose beads. Immunoprecipitated protein complexes were analyzed by immunoblotting. (*E*) HEK293 cell lysates coexpressing P-REX2ΔDHPH, IP4P, or DEPPDZ with FLAG-C2TAIL or FLAG-C2TAIL402STOP were incubated with FLAG-M2 antibody or normal mouse IgG. Immunoprecipitated protein complexes were analyzed by immunoblotting. (*F*) HEK293 cell lysates coexpressing P-REX2 or P-REX2ΔIP4P with FLAG-C2TAIL or FLAG-C2TAIL402STOP were incubated with FLAG-M2 antibody or normal mouse IgG conjugated to agarose beads. Immunoprecipitated protein complexes were analyzed by immunoblotting.

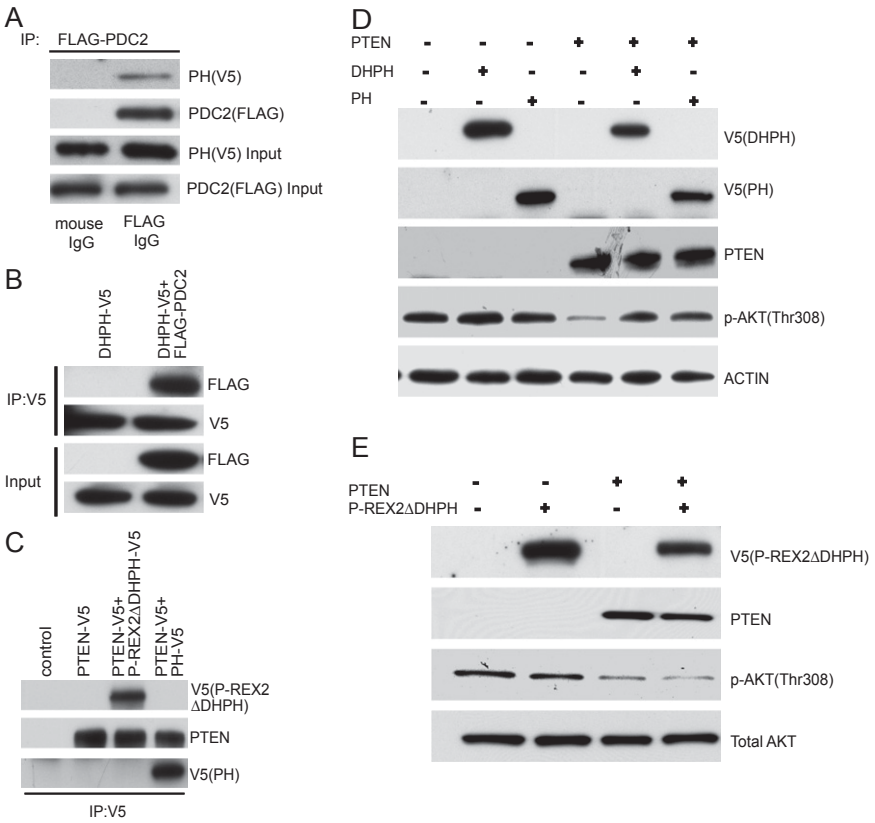

Fig. S4. The P-REX2 PH domain interacts with and inhibits the catalytic unit of PTEN. (*A* and *B*) Immunoprecipitation experiments were performed by co-transfection of PH-V5 or DHPH-V5 with FLAG-PDC2 into HEK293 cells. Protein lysates were incubated with FLAG-M2 antibody, V5-agarose beads, or normal mouse IgG conjugated to agarose beads. Immunoprecipitated proteins were analyzed by Western blot. (*C*) Representative levels of PTEN-V5, P-REX2-PH-V5, and P-REX2ΔDHPH immunoprecipitated for phosphatase assay (Fig. 3*A*). (*D* and *E*) DHPH-V5, PH-V5, or PREX2ΔDHPH-V5 and untagged PTEN were transfected alone or in combination into U87 cells. Following overnight starvation, cells were lysed and levels of pAKT-Thr308 were analyzed by Western blot.

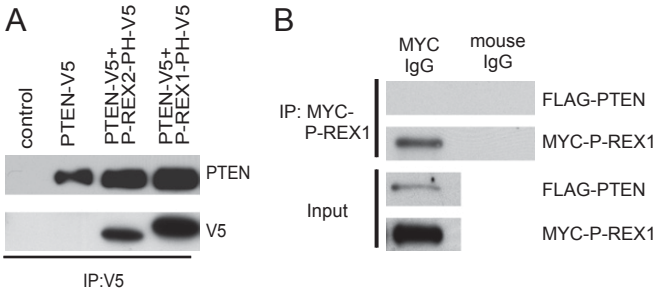

Fig. S5. Analysis of P-REX1 and PTEN interaction. (*A*) Representative levels of PTEN-V5, P-REX2-PH-V5, and P-REX1-PH-V5 immunoprecipitated for phosphatase assay (Fig. 3*B*). (*B*) FLAG-PTEN and MYC-P-REX1 were transfected into HEK293 cells. Cells were lysed and incubated with FLAG-M2 antibody or normal mouse IgG and immunoprecipitated proteins were analyzed by Western blot.

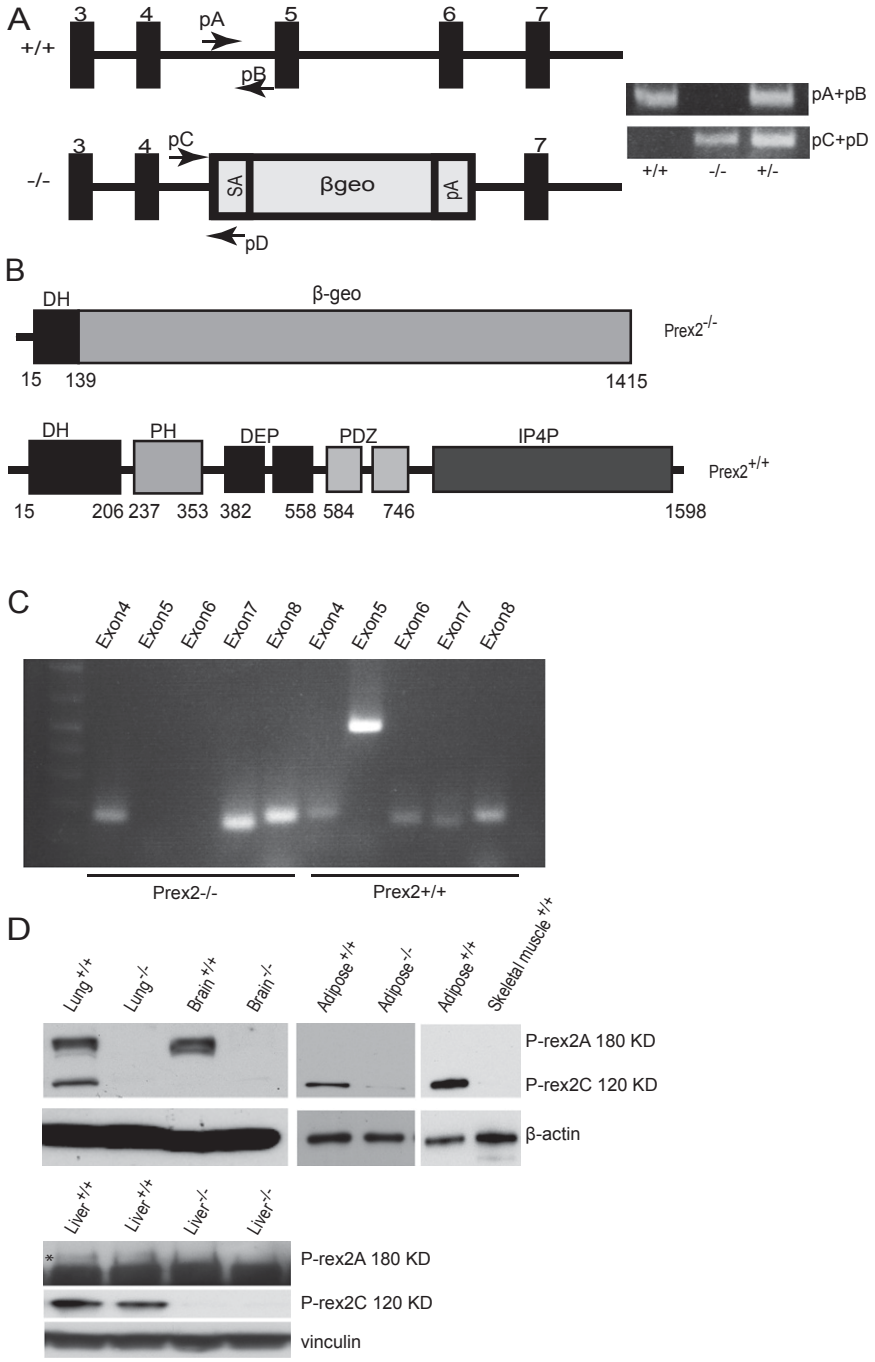

Fig. S6. Generation of *Prex2* knockout mice and expression in mouse tissue. (*A*) ES cells with a gene trap containing a splice acceptor site (SA), β-galactosidase and neomycin phosphotransferase II fusion (βgeo), and polyadenylation sequence (pA) in intron 4 were used to generate chimeras. Primers (p) used for genotyping are depicted by arrows (pA–pD), and PCR genotyping using these primers is shown. (*B* and *C*) Illustration of the *Prex2*$^{+/+}$ and *Prex2*$^{-/-}$ allele. Insertion of the βgeo reporter gene resulted in a deletion of exons 5 and 6 in the DH domain, as shown by genomic PCR. (*D*) Expression of P-rex2A (180 kDa) and P-rex2C, a shorter splice variant of 120 kDA, in various tissues. In the liver, P-rex2A protein was visualized on a 4–12% gel, whereas P-rex2C was observed on a 4% gel due to the presence of strong background bands specifically in liver lysates when using our P-REX2 antibody.

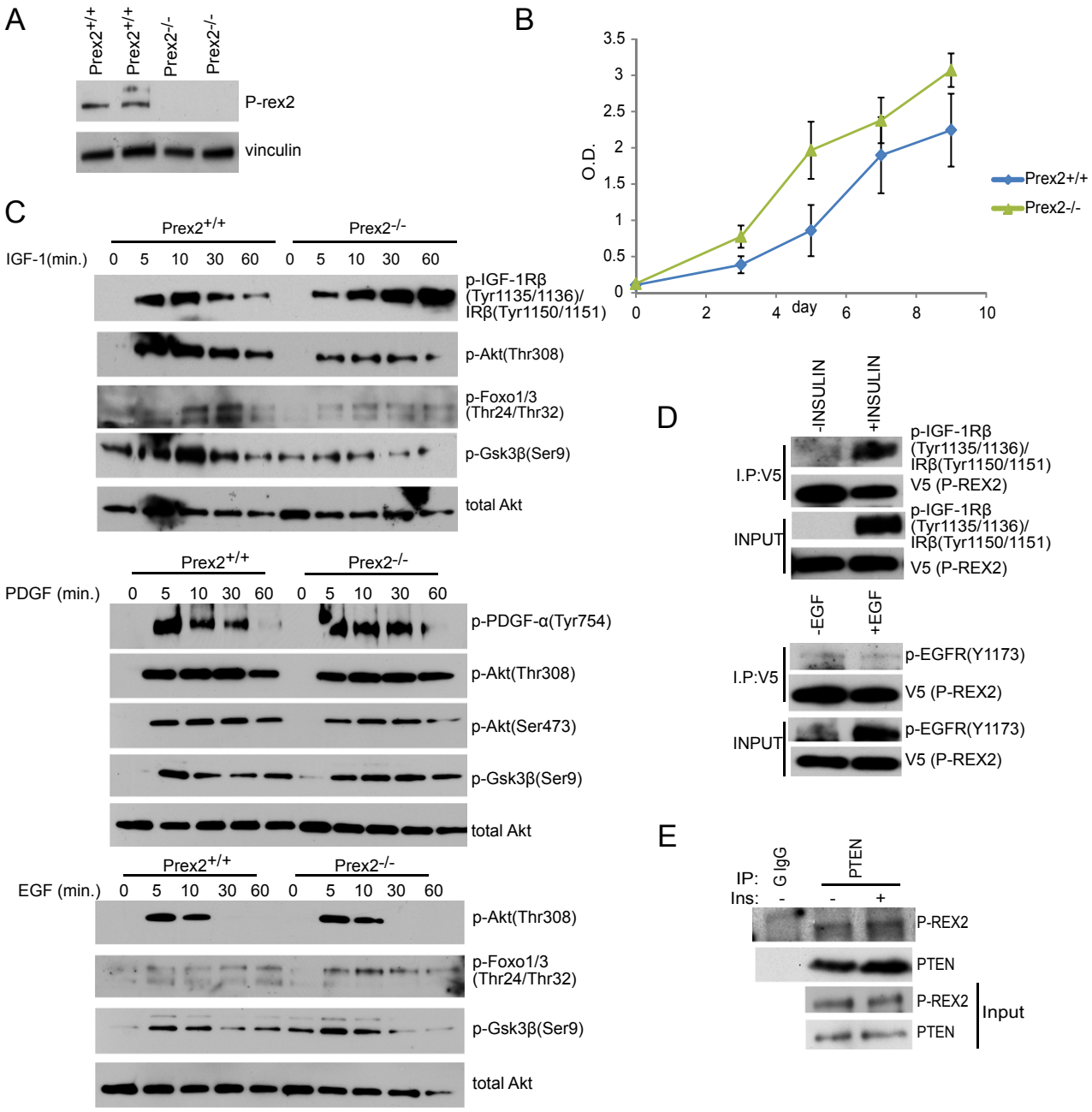

Fig. S7. Specificity of P-rex2 regulation of growth factor signaling. (*A*) Western blot analysis of P-rex2 expression in *Prex2*$^{+/+}$ and *Prex2*$^{-/-}$ MEFs. (*B*) Proliferation of *Prex2*$^{+/+}$, *Prex2*$^{+/-}$, and *Prex2*$^{-/-}$ MEFs in regular growth medium as quantified by crystal violet staining. (*C*) *Prex2*$^{+/+}$ and *Prex2*$^{-/-}$ MEFs were starved for 3 h and then stimulated with 20 ng/mL IGF1, PDGF, or EGF. Levels of phosphorylated Akt and downstream targets were analyzed over time by Western blot. (*D*) The interaction between P-REX2 and activated receptor tyrosine kinases was analyzed by coimmunoprecipitation. HEK293 cells overexpressing P-REX2-V5 were either starved overnight or stimulated with 10 μg/mL insulin or 20 ng/mL EGF for 10 min. Cells were lysed and incubated with V5-agarose beads. Total lysates and V5-immunoprecipitated receptor complexes were analyzed by Western blot using phospho-specific antibodies. (*E*) Endogenous interaction between P-rex2 and Pten in starved and stimulated *Prex2*$^{+/+}$ immortalized fibroblasts.

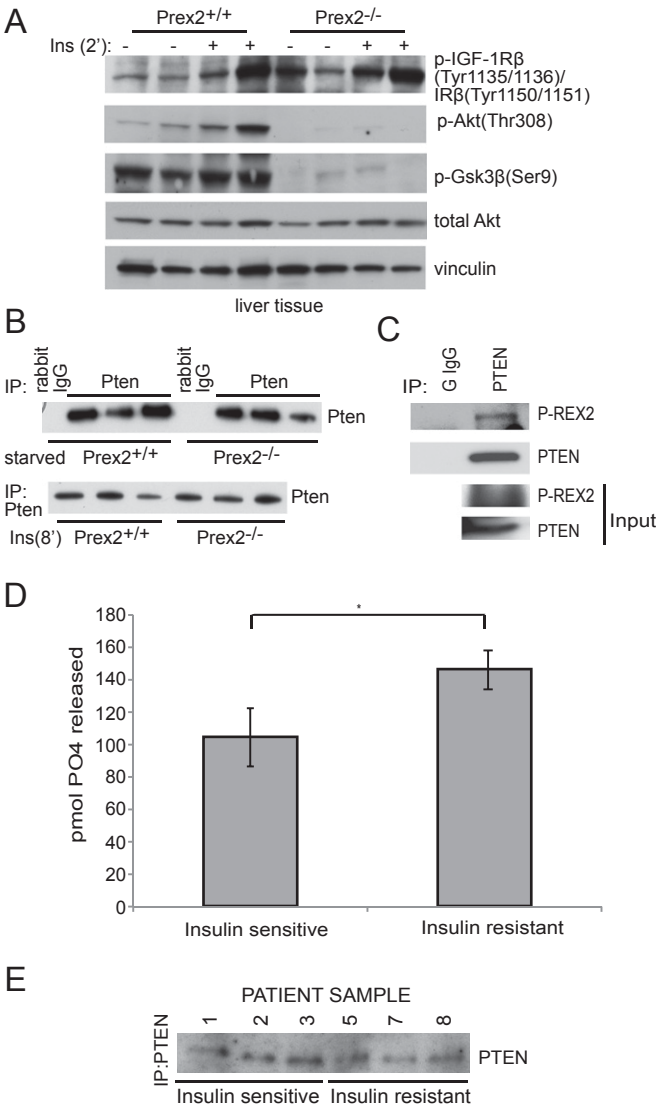

Fig. S8. Effect of *Prex2* loss in liver and adipose tissue. (*A*) *Prex2*$^{+/+}$ and *Prex2*$^{-/-}$ mice were fasted for 16 h and injected intraperitoneally with insulin (Ins, 0.75 mU/g) for 2 min. Levels of phosphorylated Akt, Gsk3β, and IRβ were analyzed by Western blot. (*B*) Representative levels of Pten immunoprecipitated from starved and insulin-stimulated liver tissue for phosphatase assays. (*C*) Endogenous coimmunoprecipitation of Pten and P-rex2 from *Prex2*$^{+/+}$ liver lysates. (*D*) Adipose tissue lysates from insulin-resistant ($n = 3$) and insulin-sensitive human subjects ($n = 3$) were incubated with PTEN antibody conjugated to agarose beads, and phosphatase assays were performed in triplicate on immunoprecipitated protein complexes. Levels of free phosphate released are shown. ±SEM, $^{*}P < 0.05$. (*E*) Representative levels of PTEN immunoprecipitated from human adipose tissue for phosphatase assays.

Table S1. Characteristics of human subjects

Subject	Sex	Age	BMI	SI
1	Female	35	22	9.9574
2	Female	44	32	4.328
3	Male	23	30	4.171
4	Female	26	31	3.383
5	Female	52	35	1.979
6	Female	38	31	1.2
7	Male	39	32	1.108
8	Male	41	32	1.032
9	Female	22	31	1.0268

Sex, age, body mass index (BMI), and clinically determined insulin sensitivity index (SI) of human subjects from which s.c. adipose tissue biopsies were obtained. Samples are listed from highest insulin responders to lowest insulin responders.