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Regulation of Insulin Receptor Signaling by the Protein Tyrosine Phosphatase TCPTP

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The human protein tyrosine phosphatase TCPTP exists as two forms: an endoplasmic reticulum-targeted 48-kDa form (TC48) and a nuclear 45-kDa form (TC45). Although targeted to the nucleus, TC45 can exit in response to specific stimuli to dephosphorylate cytoplasmic substrates. In this study, we investigated the downregulation of insulin receptor (IR) signaling by TCPTP. In response to insulin stimulation, the TC48-D182A and TC45-D182A "substrate-trapping" mutants formed stable complexes with the endogenous tyrosine-phosphorylated IR β-subunit in 293 cells. Moreover, in response to insulin stimulation, the TC45-D182A mutant accumulated in the cytoplasm of cells overexpressing the IR and in part colocalized with the IR β-subunit at the cell periphery. These results indicate that the IR may serve as a cellular substrate for both TC48 and TC45. In immortalized TCPTP$^{-/-}$ murine embryo fibroblasts, insulin-induced IR β-subunit tyrosine phosphorylation and protein kinase PKB/Akt activation were enhanced relative to the values in TCPTP$^{+/+}$ cells. Importantly, the expression of TC45 or TC48 to physiological levels suppressed the enhanced insulin-induced signaling in TCPTP$^{-/-}$ cells. These results indicate that the differentially localized variants of TCPTP may dephosphorylate the IR and downregulate insulin-induced signaling in vivo.

Insulin binding to its cell surface transmembrane receptor stimulates intrinsic protein tyrosine kinase activity, autophosphorylation, and subsequent tyrosyl phosphorylation of insulin receptor substrate (IRS) proteins and adapter proteins such as Shc and Cbl/CAP (reviewed in references 5, 40, 54, and 55). Tyrosyl phosphorylation of IRS and adapter proteins generates docking sites for src homology 2 (SH2) domain-containing signaling proteins such as the p85 subunit of phosphatidylinositol 3-kinase. Collectively, these signaling proteins mediate the biological effects of insulin, which include antilipolysis, glucose uptake, glycogen synthesis, and cell growth (reviewed in references 5, 40, 54, and 55).

Protein tyrosine phosphatases (PTPs) have a prominent role in the control of insulin receptor (IR) signaling. PTPs dephosphorylate the IR and its substrates and thus serve to inactivate the IR and terminate signaling. The IR and IRS proteins are rapidly and transiently phosphorylated in response to insulin, with phosphotyrosine (pTyr) content in the IR returning to basal levels within minutes of stimulation (10, 16). It is probable that numerous PTPs participate in the dephosphorylation of the IR and the multiple downstream tyrosine-phosphorylated signaling proteins. Several PTPs have been implicated in the negative regulation of the IR, including the endoplasmic reticulum-associated PTP1B (6, 8, 49, 50) and the transmembrane PTPs LAR (34) and PTPα (30, 41). PTPα has been shown to inhibit insulin-induced prolactin gene expression without altering the phosphorylation status of the IR, IRS-1, or Shc (26). Similarly, LAR may not dephosphorylate the IR in vivo but may downregulate insulin signaling by dephosphorylating adaptor proteins such as IRS-2 (38, 42, 59).

Over the past several years, numerous reports have provided conclusive evidence for PTP1B's role in IR receptor signaling. PTP1B can dephosphorylate the IR in vitro, and with substrate-trapping mutants, several studies have demonstrated that PTP1B can recognize the IR as a cellular substrate (reviewed in reference 51). Moreover, ectopic expression of PTP1B in cells can inhibit insulin signaling, whereas impairment of PTP1B function stimulates insulin signaling (reviewed in reference 51). The most compelling evidence for PTP1B's role in IR signaling has come from Elchebly et al. (12) and Klaman et al. (28), who report that mice lacking PTP1B have increased insulin sensitivity and resistance to obesity induced by a high-fat diet. Although PTP1B-null mice exhibit enhanced IR tyrosine phosphorylation in muscle (approximately 1.75-fold at 2 min following bolus administration of insulin to the inferior vena cava) and sustained IR tyrosine phosphorylation in the liver (12), the IR is nevertheless eventually dephosphorylated (51). Moreover, insulin sensitivity in PTP1B-dificient mice is tissue specific, and although PTP1B has been implicated in the regulation of IR in liver and skeletal muscle, it does not have a role in the regulation of IR in adipose tissue (12, 28). Consistent with in vivo data, overexpressed PTP1B inhibits IR signaling and glucose uptake in L6 myocytes and Fao hepatoma cells but has no effect in 3T3-L1 adipocytes (11, 52). Thus, not only must additional PTPs participate in the downregulation of IR in liver and skeletal muscle, but an altogether different PTP must regulate IR tyrosine phosphorylation in adipocytes.

All PTPs have a conserved catalytic domain of approximately 280 residues sharing a sequence identity of approximately 35% (1, 4). Human TCPTP is the PTP most closely

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related to PTP1B (1), with 72% sequence identity and 86% similarity in the catalytic domain (residues 43 to 288). A comparison of the crystal structure of the TCPTP catalytic domain (residues 5 to 277) (25) to that of PTP1B (3) indicates that the two phosphatases have virtually identical catalytic active sites (25). Recently, the crystal structure of PTP1B in complex with the IR β-subunit activation loop peptide encompassing the tandem phosphotyrosine (pTyr) residues at 1162 and 1163 was solved (39). In this structure, whereas pTyr-1162 was selected at the PTP1B catalytic active site, pTyr-1163 was bound to an adjacent pTyr recognition pocket and increased PTP1B's selectivity for the tandemly phosphorylated IR peptide (39). The side chains from Arg-24 and Arg-254 from this second pTyr recognition pocket formed salt bridges directly with the phosphate of pTyr-1163 (39).

Although Arg-254 is invariant within the PTP family, TCPTP and PTP1B are the only known PTPs that possess Arg-24. In addition, the crystal structure revealed that access to the second pTyr recognition pocket by pTyr-1163 was made possible by the small side chain of Gly-259. Gly-259 is present in TCPTP, but in most other PTPs, including all receptor PTPs, Gly-259 is replaced by amino acids with bulky side chains that would sterically hinder the access of pTyr-1163 (39). Given the conservation of the second pTyr recognition pocket (25), it is apparent that the TCPTP catalytic domain at least has the potential to recognize the tandemly phosphorylated IR similarly to PTP1B and moreover that such recognition of the IR may be unique to PTP1B and TCPTP.

Two forms of human TCPTP exist that have identical catalytic domains but different noncatalytic C-termini: a 48-kDa form (TC48) that is targeted to the endoplasmic reticulum by a stretch of hydrophobic residues at the extreme C terminus, and a 45-kDa variant (TC45) that lacks the hydrophobic C-terminal tail and is targeted to the nucleus by an atypical bipartite nuclear localization sequence (22, 33, 47). Although targeted to the nucleus, TC45 can exit the nucleus in response to specific stimuli to dephosphorylate distinct substrates and regulate specific signaling processes (45, 46, 48). Several cellular substrates of TCPTP have been identified. These include the epidermal growth factor (EGF) receptor, the adaptor protein $p52^{Shc}$ (29, 46, 48), the Janus family kinases (JAKs) 1 and 3 (43), and signal transducer and activator of transcription (STAT) 1 (44, 60). Previous overexpression studies have shown that the endoplasmic reticulum-localized TC48 can dephosphorylate the overexpressed IR in 293 cells (32). More recent studies have shown that the recombinant TCPTP catalytic domain can dephosphorylate the IR in vitro as well as PTP1B (53). Here we have used TCPTP substrate-trapping mutants and TCPTP-null cells and identified the IR as an in vivo substrate for both TC48 and TC45.

MATERIALS AND METHODS

Materials. Insulin from bovine pancreas and Ham's F12 medium were purchased from Sigma (St. Louis, Mo.). Monoclonal IR β-subunit (Ab-6) antibody, used for immunoblotting and immunofluorescence, and the IRS-1 (Ab-1) antibody were purchased from NeoMarkers (Fremont, Calif.); polyclonal IR β-subunit dual-phospho-specific (pYpY1162/1163) antibodies from BioSource International (Camarillo, Calif.); monoclonal IR β-subunit antibodies used for immunoprecipitation were from BD Transduction Laboratories (San Diego, Calif.) and Santa Cruz Biotechnology (29B4, SC-09; Santa Cruz, Calif.); polyclonal phospho-Akt (Ser473) and Akt antibodies were from New England Biolabs (Beverly, Mass.); and goat antiactin (SC-1616) was from Santa Cruz Biotechnology (Santa Cruz, Calif.). The following reagents were supplied generously by colleagues: monoclonal antibodies anti-TCPTP CF4 and anti-PTP1B FG6 by D. Hill (Calbiochem Oncogene Research Products, Cambridge, Mass.), monoclonal TCPTP antibody 3E2 (23) by M. L. Tremblay (McGill University, Quebec, Canada), and polyclonal 6228 TCPTP antibodies (33) by E. H. Fischer (University of Washington, Seattle). Monoclonal pTyr G98 (subtype immunoglobulin M; used for immunoblotting) and G104 (subtype immunoglobulin G; used for immunoprecipitation) antibodies have been described previously (14, 46). We are most grateful to Michel L. Tremblay (McGill University, Quebec Canada) and Kinetek Pharmaceuticals Inc. (Vancouver, British Columbia, Canada) for providing the $TCPTP^{-/-}$ cell lines $EFM4^{-/-}$ and $EFM14^{-/-}$ and the $TCPTP^{+/+}$ cell lines $EFM7^{+/+}$ and $EFM11^{+/+}$ (23).

Plasmid constructs. TC48-pBluescript II KS(+), TC45-pWZL(Hygro), and pMT2 and pCG constructs encoding TC45 and TC45-D182A have been described previously (29, 31, 47). To generate the TC48-pWZL(Hygro) construct, human TC48 cDNA was excised with *Sal*I and *Eco*RI from the TC48-pBluescript II KS(+) construct and cloned into the same sites of the retroviral expression vector pWZL(Hygro). The structure of the recombinant plasmid was confirmed by restriction endonuclease analysis.

Reverse transcription-PCR analysis. Total RNA from $TCPTP^{-/-}$ ($EFM4^{-/-}$) or $TCPTP^{+/+}$ ($EFM7^{+/+}$) cells was isolated with the RNAeasy kit (Qiagen GmbH, Germany); 6 μg of RNA, oligo$(dT)_{15}$ primer (Promega, Madison, Wis.), and avian myeloblastosis virus reverse transcriptase (Roche Diagnostics GmbH, Mannheim, Germany) were used for first-strand cDNA synthesis for 3 h according to the manufacturer's instructions. One fifth of this reaction mix was used for the amplification of TCPTP cDNAs by PCR with recombinant *Taq* DNA polymerase (Invitrogen, Carlsbad, Calif.) and 30 ng of each of the forward and reverse oligonucleotide primers in a 25-μl reaction. Amplification parameters were 5 min at 94°C, 40 cycles of 1 min at 94°C, 1 min at 50°C, and 1 min at 72°C, followed by an additional 10 min at 72°C. The forward primer for murine TCPTP was 5′-AGCAGTTGTCATGCTGAA-3′ and the reverse primer was 5′-TGGACACTGCTTTTTCACTAA-3′ for TC48 and 5′-AGGCCAAGATTGACAGACACCTAA-3 for TC45. β-Actin primers were 5′-TGAAGTCTGACGTGGACATC-3′ and 5′-ACTCGTCATACTCCTGCTTG-3′.

Cell culture. Chinese hamster ovary (CHO) cells stably overexpressing the human insulin receptor (CHO/IR) were cultured at 37°C and 5% CO_2 in Ham's F12 medium containing 10% fetal bovine serum, 100 U of penicillin per ml, and 100 μg of streptomycin per ml. Human embryonic kidney 293 cells and the $TCPTP^{-/-}$ and $TCPTP^{+/+}$ murine embryo fibroblast cell lines ($EFM4^{-/-}$ $EFM14^{-/-}$, $EFM7^{+/+}$, and $EFM11^{+/+}$) were cultured under the same conditions in Dulbecco's modified Eagle's medium containing 10% (vol/vol) fetal bovine serum plus antibiotics. Where indicated, cells were serum starved in medium containing 0.1% (vol/vol) fetal bovine serum plus antibiotics. CHO/IR and 293 cells were serum starved for 20 h, whereas $TCPTP^{-/-}$ and $TCPTP^{+/+}$ cells were serum starved for 4 to 5 h.

TCPTP reconstitution in TCPTP-null cells. Retroviruses encoding human TC48 or TC45 were generated as described previously (15). Briefly, the BING replication-incompetent virus packaging cell line was electroporated with either TC48-pWZL(Hygro) or TC45-pWZL(Hygro) DNA. Virus-containing supernatants were harvested and added to cultures of $TCPTP^{-/-}$ cells ($EFM4^{-/-}$). Drug-resistant colonies were isolated after 7 days of selection in medium containing 100 μg of hygromycin B (Invitrogen, Carlsbad, Calif.) per ml and maintained in the same medium. All experiments were performed in the absence of hygromycin B.

Transfections and immunoprecipitations. 293 cells were electroporated as described previously (29) with either 20 μg of pCG vector control, TC45-pCG, or TC45-D182A-pCG or 40 μg of TC48-pMT2 or TC48-D182A-pMT2. The efficiency of electroporation, as assessed at 24 h by 5-bromo-4-chloro-3-indolyl β-D-galactosidase (X-Gal) staining of pCMV-β-galactosidase-electroporated cells, was routinely 75 to 90%. At 24 h posttransfection the electroporated cells were serum starved for 20 h and either left untreated or stimulated with 100 nM insulin for 15 min. Cells were then lysed in immunoprecipitation lysis buffer (50 mM Tris [pH 7.5], 1% [wt/vol] NP-40, 150 mM NaCl, 50 mM NaF, leupeptin [5 μg/ml], pepstatin [1 μg/ml], 1 mM benzamidine, 2 mM phenylmethylsulfonyl fluoride) and processed for TCPTP immunoprecipitation with the CF4 antibody in the presence of 5 mM iodoacetic acid as described previously (46).

pTyr-containing proteins from $TCPTP^{-/-}$ ($EFM4^{-/-}$) and $TCPTP^{+/+}$ ($EFM7^{+/+}$) cells that had been serum starved or serum starved and insulin stimulated (10 nM, 45 to 60 min) were immunoprecipitated from 1 mg of total protein (as determined by the method of Bradford) in the presence of 1 mM sodium orthovanadate with anti-pTyr G104 antibody (10 μl of ascites) for 2 h at 4°C under constant mixing. Immune complexes were collected on protein A-

Sepharose CL-4B (Pharmacia, Uppsala, Sweden) for 60 min at 4°C and washed four times with immunoprecipitation lysis buffer containing 2 mM sodium orthovanadate and once with 50 mM Tris, pH 7.5. Immune complexes were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotted as indicated.

Immunofluorescence. CHO/IR cells were electroporated as described previously (56). Briefly, the equivalent of one 10-cm dish of 80 to 90% confluent CHO/IR cells was electroporated with 30 μg of pMT2 vector control, TC45-pMT2, or TC45-D182A-pMT2 plasmid (46) at 340 V and 975 μF in 0.4-cm cuvettes and seeded onto glass coverslips in a 6-cm dish containing 5 ml of medium. At 2 to 3 h posttransfection, cells were supplemented with fresh medium. After serum starvation and stimulation with insulin (100 nM, 30 min), the cells were fixed with 3.2% (wt/vol) paraformaldehyde in phosphate-buffered saline and processed as described previously (46) with the monoclonal anti-TCPTP antibody CF4 or polyclonal anti-TCPTP 6228 and monoclonal anti-IR β-subunit antibodies.

RESULTS

TCPTP-D182A substrate-trapping mutants can form complexes with tyrosine-phosphorylated IR β-subunit. All PTPs contain a conserved Asp that serves as a general acid catalyst, and mutation of this residue (Asp 182 in TCPTP) to Ala generates mutants that can form complexes with tyrosine-phosphorylated substrates in a cellular context (13, 46). To ascertain whether TC48 and TC45 may recognize the IR as a cellular substrate, we compared the abilities of the respective D182A substrate-trapping mutants to form complexes with the tyrosine-phosphorylated IR β-subunit. TC48 and TC45 and the respective D182A mutants were expressed transiently in 293 cells, which express IR endogenously. Following serum starvation and stimulation with 100 nM insulin for 15 min, the overexpressed TCPTP proteins were immunoprecipitated and the association of tyrosine-phosphorylated IR β-subunit was assessed by immunoblot analysis (Fig. 1).

Tyrosine-phosphorylated IR β-subunit association was monitored with antibodies specific for the IR β-subunit activation loop phosphorylated on the tandem tyrosine residues at 1162 and 1163 (Fig. 1A) and antibodies specific for pTyr (Fig. 1B). IR β-subunit phosphorylated on tyrosine and specifically on tyrosines 1162 and 1163 could be coimmunoprecipitated with the TC48-D182A and TC45-D182A mutants but not the wild-type TC48 or TC45 in response to insulin (Fig. 1). Moreover, the D182A/pTyr-IR β-subunit complexes could be disrupted by the addition of the PTP active site-directed inhibitor vanadate (data not shown), indicating that in each case, the D182A trapping mutant and the pTyr-IR β-subunit were interacting through the PTP active site. These results indicate that both TC48 and TC45 have the propensity to recognize endogenous IR in cells and are consistent with the IR β-subunit's being a direct substrate for TCPTP. Notably, with the exception of a nonspecific pTyr protein that was also present in vector control immunoprecipitates in both the absence and presence of insulin, no other pTyr-containing proteins were detected in the D182A immunoprecipitates (Fig. 1B), indicating that the IR β-subunit is a major insulin-induced TC48 and TC45 substrate in 293 cells.

TC45-D182A substrate-trapping mutant accumulates in the cytoplasm following insulin stimulation. We have reported previously that under basal conditions, TC45 is nuclear, but in response to specific stimuli, TC45 can shuttle out of the nucleus to regulate cellular signaling in the cytoplasm (31, 46, 48). This change in localization can be visualized in cells overexpressing the TC45-D182A substrate-trapping mutant, which can form stable complexes with tyrosine-phosphorylated cytoplasmic substrates, resulting in accumulation of the TC45-D182A mutant in the cytoplasm (46). For example, in response to EGF stimulation, the TC45-D182A mutant forms a complex and colocalizes with the EGF receptor at the cell periphery (46, 48).

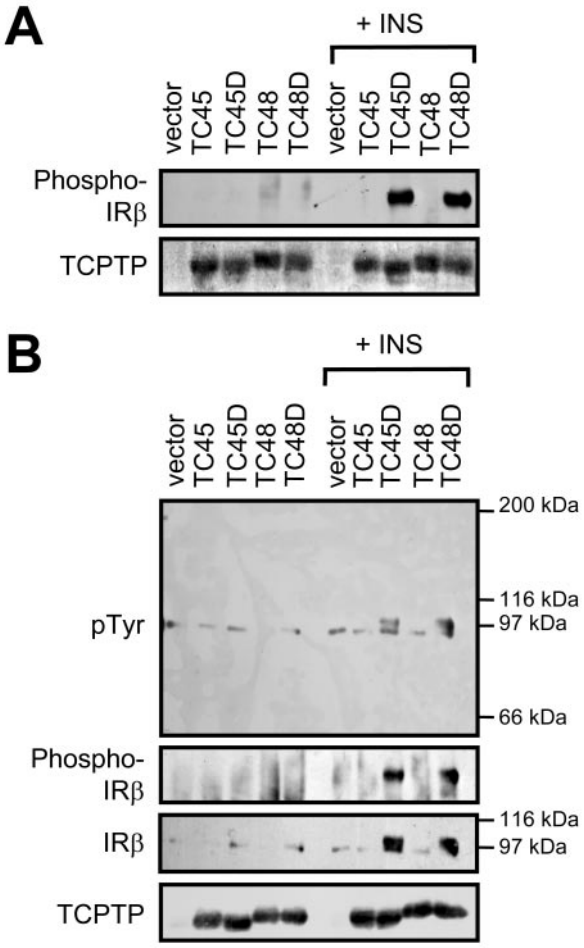


FIG. 1. TCPTP recognizes the tyrosine-phosphorylated IR β-subunit as a cellular substrate. 293 cells transfected with vector control or plasmids expressing TC45, TCA5-D182A (TC45D), TC48, or TC48-D182A (TC48D) were serum starved and stimulated with 100 nM insulin (INS) for 15 min. (A) TCPTP immunoprecipitates were resolved by SDS-PAGE and immunoblotted with polyclonal antibodies specific for the IR β-subunit phosphorylated on tyrosines 1162 and 1163 (phospho-IR) or TCPTP. (B) TCPTP immunoprecipitates were resolved and immunoblotted with antibodies specific for pTyr and reprobed for phospho-IR. Immunoblots were then stripped as described previously (46) and reprobed with antibodies specific for the IR β-subunit (IRβ) or TCPTP.

To determine whether TC45 exits the nucleus in response to insulin stimulation, we ascertained the subcellular localization of the TC45-D182A substrate-trapping mutant in CHO cells stably expressing the IR (CHO/IR). CHO/IR cells overexpressing the TC45-D182A mutant were serum starved and either left untreated or stimulated with insulin and then processed for immunofluorescence microscopy with antibodies specific for TCPTP or the IR β-subunit (Fig. 2). Under serum starvation

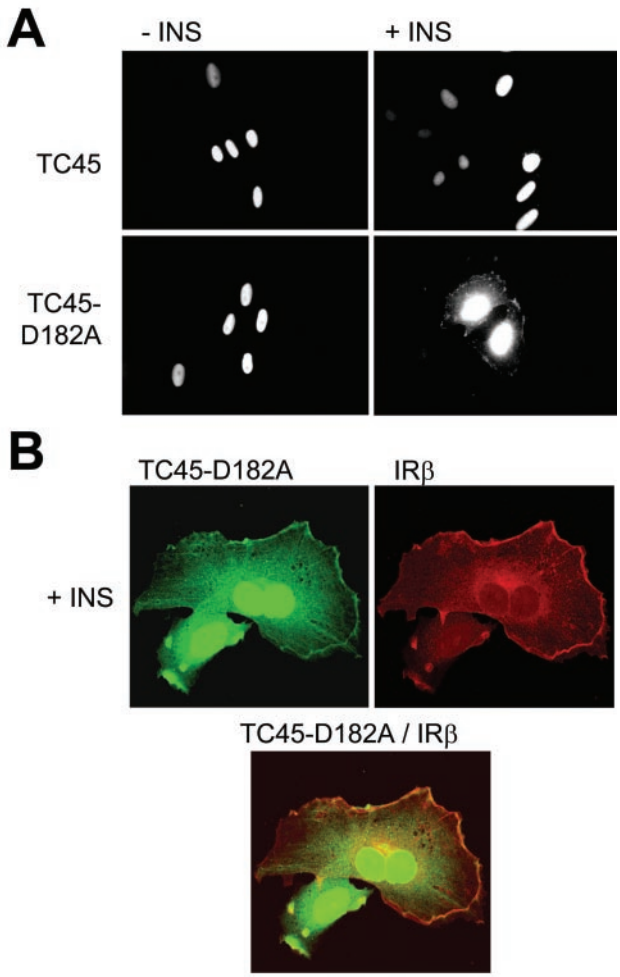


FIG. 2. TC45-D182A exits the nucleus and colocalizes with IR β-subunit in response to insulin. CHO/IR cells were transfected with pCG constructs expressing wild-type TC45 or the TC45-D182A substrate-trapping mutant (TC45D). At 24 h posttransfection, cells were serum starved and either left untreated or stimulated with 100 nM insulin (INS) for 30 min. Cells were processed for (A) epifluorescence microscopy with the monoclonal anti-TCPTP antibody CF4 or (B) for confocal microscopy with the rabbit polyclonal anti-TCPTP antibody 6228 and a mouse monoclonal IR β-subunit (IRβ) antibody as described previously (48).

conditions, TC45-D182A localized to the nucleus (Fig. 2A). After stimulation with insulin, TC45-D182A accumulated in the cytoplasm (Fig. 2A) and in part colocalized with the IR β-subunit at the cell periphery (Fig. 2B). These results indicate that TC45 can exit the nucleus and have access to the tyrosine-phosphorylated IR in the cytoplasm and at the cell periphery in response to insulin stimulation.

Insulin-induced IR β-subunit tyrosine phosphorylation is enhanced in TCPTP-null cells. To ascertain TCPTP's potential to dephosphorylate the IR in vivo, insulin-induced IR activation and signaling were compared in cell lines established previously by spontaneous immortalization of TCPTP$^{-/-}$ and TCPTP$^{+/+}$ primary mouse embryo fibroblasts (23). As expected, TCPTP-null cells (EFM4$^{-/-}$) lacked TCPTP but expressed similar amounts of the IR β-subunit, IRS-1, the p85

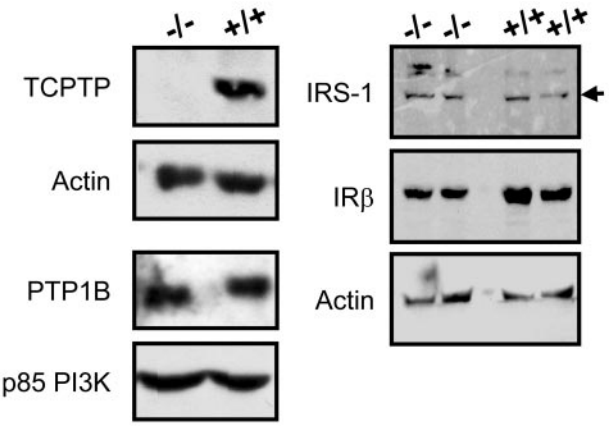
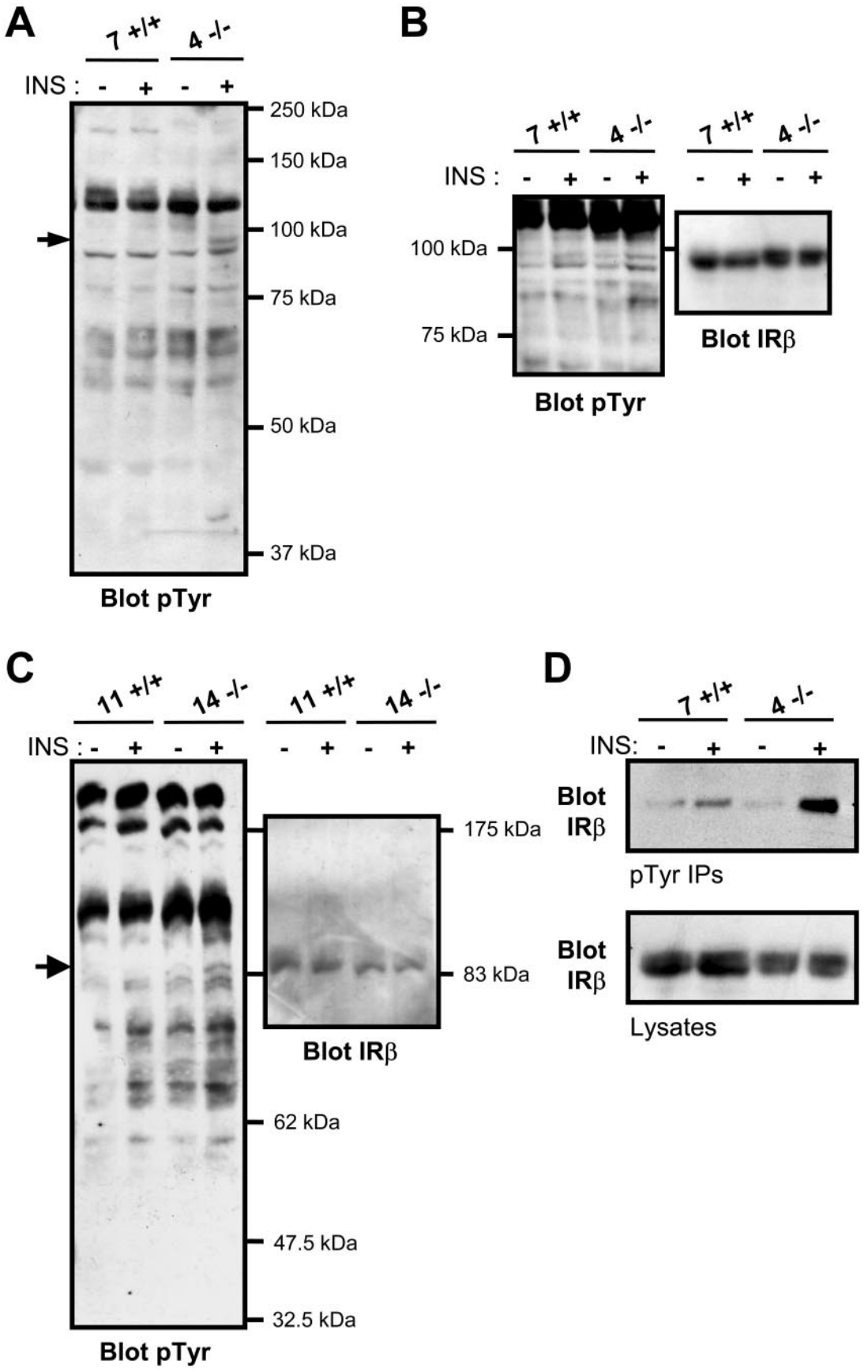


FIG. 3. Expression of signaling proteins mediating insulin action in TCPTP$^{-/-}$ versus TCPTP$^{+/+}$ cells. Equal amounts of protein from TCPTP$^{-/-}$ (EFM4$^{-/-}$) and TCPTP$^{+/+}$ (EFM7$^{+/+}$) cells were resolved on SDS-PAGE and immunoblotted with anti-TCPTP 3E2 antibody (23) or antibodies specific for PTP1B, the p85 regulatory subunit of phosphatidylinositol 3-kinase (PI3K), IR β-subunit (IRβ), IRS-1 (indicated by arrow), or actin.

subunit of phosphatidylinositol 3-kinase, PTP1B (Fig. 3), and PKB/Akt (data not shown), indicating that the cells should otherwise be comparable in their ability to transduce signaling in response to insulin. We assessed the tyrosine phosphorylation state of proteins in lysates from TCPTP$^{-/-}$ (EFM4$^{-/-}$) versus TCPTP$^{+/+}$ (EFM7$^{+/+}$) cells following insulin stimulation by immunoblot analysis with pTyr-specific antibodies (Fig. 4A).

Strikingly, the change in the pattern of tyrosine-phosphorylated proteins in the TCPTP$^{-/-}$ cells was subtle, attesting to the specificity of TCPTP (Fig. 4A). Two insulin-induced pTyr-containing proteins with apparent molecular masses of approximately 42 kDa and 95 kDa were present in the TCPTP-null but not in wild-type cell lysates (Fig. 4A). The ≈42-kDa pTyr protein was identified as the mitogen-activated protein kinase ERK2 by immunoblotting with antibodies specific for ERK2 protein or the phosphorylated and activated ERK1/2 (data not shown). Based on molecular weight, we speculated that the other insulin-induced pTyr protein in the TCPTP$^{-/-}$ cell lysates might be the IR β-subunit (95 kDa). Consistent with this supposition, we found that the IR β-subunit comigrated with the ≈95-kDa pTyr protein (Fig. 4B). Similar results for the ≈95-kDa pTyr protein were acquired with a second independent pair of immortalized mouse embryo fibroblast TCPTP$^{-/-}$ (EFM14$^{-/-}$) and TCPTP$^{+/+}$ (EFM11$^{+/+}$) cells (Fig. 4C). Here too the ≈95-kDa pTyr protein comigrated with the IR (Fig. 4C), indicating that IR-pTyr may be enhanced in TCPTP-null cells. In contrast, the ≈42-kDa pTyr protein was not observed in EFM14$^{-/-}$ cells (Fig. 4A), indicating that the enhanced ERK1/2 activation in EFM4$^{-/-}$ cells (Fig. 4C) may be attributed to cell line differences arising from immortalization rather than TCPTP loss.

The TCPTP$^{-/-}$ and TCPTP$^{+/+}$ cells expressed very small amounts of the IR, and by using two commercially available antibodies that otherwise immunoprecipitated the IR β-subunit from differentiated 3T3-L1 adipocytes and HepG2 hepatocytes, we were unable to immunoprecipitate the IR β-sub-


A
7 +/+
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B
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unit from TCPTP$^{-/-}$ or TCPTP$^{+/+}$ cells. As such, we could not investigate directly the tyrosine phosphorylation state of the IR β-subunit in IR immunoprecipitates. To circumvent this technical difficulty, we monitored for the presence of the IR β-subunit in pTyr immunoprecipitates from serum-starved and insulin-stimulated TCPTP$^{-/-}$ (EFM4$^{-/-}$) versus TCPTP$^{+/+}$ (EFM7$^{+/+}$) cells (Fig. 4D). Similar amounts of the IR β-subunit protein were present in the lysates utilized for the pTyr immunoprecipitations (Fig. 4D), and given the comparable levels of overall pTyr in TCPTP$^{-/-}$ versus TCPTP$^{+/+}$ cells (Fig. 4A), we reasoned that any differences in IR β-subunit association should reflect differences in IR β-subunit pTyr status. We found significantly higher amounts of IR β-subunit protein in pTyr immunoprecipitates from TCPTP$^{-/-}$ compared to TCPTP$^{+/+}$ cells stimulated with 10 nM insulin for 5 min (data not shown) or 60 min (Fig. 4D). Taken together, these results indicate that the IR β-subunit is hyperphosphorylated in TCPTP-null cells following insulin stimulation.

Insulin-induced signaling is enhanced in TCPTP-null cells. Tyrosine phosphorylation of molecules by the IR creates docking sites for the recruitment of SH2 and PTB domain-containing proteins for the activation of downstream signaling cascades (reviewed in references 5, 40, 54, and 55). These signaling processes include the phosphatidylinositol 3-kinase/protein kinase PKB/Akt pathway that can stimulate GLUT4 translocation and glucose uptake in muscle and adipose tissue (9, 21; reviewed in references 5, 40, 54, and 55). We determined the status of PKB/Akt activation in TCPTP$^{-/-}$ (EFM4$^{-/-}$) relative to TCPTP$^{+/+}$ (EFM7$^{+/+}$) cells following stimulation with 1 nM (Fig. 5A) or 10 nM (Fig. 5B) insulin with antibodies specific for the phosphorylated and activated PKB/Akt (phospho-Akt) (Fig. 5A and B). When comparing TCPTP$^{-/-}$ to TCPTP$^{+/+}$ cells, insulin stimulations were undertaken at the same time and proteins from the respective cell lines were resolved by SDS-PAGE and transferred onto the same membrane for immunoblot analysis.

Phospho-Akt immunoblots were quantitated by densitometric analysis and normalized for total PKB/Akt, and the kinetics of PKB/Akt activation were compared. We found that after stimulation with 1 nM insulin, PKB/Akt activity declined slowly after the maximum at 5 to 15 min in TCPTP$^{-/-}$ cells and remained activated even at 45 to 60 min (Fig. 5A). In contrast, in TCPTP wild-type cells, PKB/Akt activity was maximal at 5 to 10 min and declined rapidly thereafter, such that it was negligible at 30 min (Fig. 5A). In response to 10 nM insulin, PKB/Akt activity in TCPTP-null cells was maximal within 2 min and sustained for up to 45 min (Fig. 5B). In contrast, in TCPTP$^{+/+}$ cells, PKB/Akt activity was maximal at 5 min and declined rapidly after 10 min (Fig. 5B). These results indicate that the loss of TCPTP results in enhanced insulin-induced PKB/Akt signaling.

TCPTP reconstitution suppresses the enhanced and prolonged IR β-subunit tyrosine phosphorylation and signaling in TCPTP-null cells. To provide further evidence that TCPTP loss results in enhanced insulin-induced signaling, we decided to reconstitute TCPTP in TCPTP$^{-/-}$ cells and assess whether IR β-subunit pTyr and PKB/Akt activation were altered. However, first we determined whether TC48, TC45, or both were expressed in the TCPTP$^{+/+}$ cells. Previous studies have reported that TC48 is not detectable in TCPTP$^{+/+}$ cells (23, 43, 44). By immunoblot analysis with either monoclonal antibody 3E2 (which recognizes a region in the TCPTP noncatalytic C terminus that is common to both TC48 and TC45) (Fig. 3) or polyclonal 6228 antibodies (which recognize the TCPTP catalytic domain), we could only detect a single protein in wild-type cells that was not present in TCPTP$^{-/-}$ cells (Fig. 6A). This protein was approximately 45 kDa (Fig. 6A), and based on this molecular mass, it would be reasonable to conclude that the major TCPTP variant in TCPTP$^{+/+}$ cells was TC45. To assess whether TC48 was expressed at all in TCPTP$^{+/+}$ cells, we used reverse transcription-PCR and oligonucleotide primers specific for either murine TC48 or TC45 (Fig. 6B) and examined TCPTP mRNA expression. mRNA for both TC48 and TC45 was detected in TCPTP$^{+/+}$ (EFM7$^{+/+}$) but not in TCPTP$^{-/-}$ (EFM4$^{-/-}$) cells (Fig. 6C). These results indicate that although TC45 may predominate in TCPTP$^{+/+}$ cells, TC48 protein may still be present, albeit at significantly lower levels.

We stably reconstituted either TC48 or TC45 in the TCPTP$^{-/-}$ cells with retroviruses and isolated individual clones for assessment of IR β-subunit tyrosine phosphorylation and insulin-induced signaling. At least three TC45 (denoted 45-R5, 45-R19, and 45-R20) and three TC48 (denoted 48-R9, 48-R11, and 48-R12) reconstituted clones were chosen for analysis (Fig. 6D). These clones expressed TC45 or TC48 to levels that were roughly 30% to 100% of those for total TCPTP in TCPTP$^{+/+}$ cells, as assessed by Western blot analysis (Fig. 6D). The tyrosine phosphorylation state of the IR in the TC45-reconstituted cells was compared to that of TCPTP-null cells. We observed that TC45 reconstitution suppressed the tyrosine phosphorylation of the 95-kDa protein that comigrated with the IR β-subunit in cell lysates (data not shown). Consistent with this, the reconstitution of TC45 also suppressed the en-

FIG. 4. Insulin-induced tyrosine phosphorylation in TCPTP$^{-/-}$ versus TCPTP$^{+/+}$ cells. (A) TCPTP$^{-/-}$ (EFM4$^{-/-}$) or TCPTP$^{+/+}$ (EFM7$^{+/+}$) cells were either left untreated or stimulated with 100 nM insulin (INS) for 10 min. Cells were lysed, and proteins were resolved on SDS-PAGE and immunoblotted with phosphotyrosine (pTyr)-specific antibodies. Molecular mass standards (precision prestained standards; Bio-Rad, Hercules, Calif.) are indicated on the right, and an arrow on the left highlights the ≈95-kDa pTyr protein in TCPTP$^{-/-}$ cells following insulin stimulation. (B) TCPTP$^{-/-}$ (EFM4$^{-/-}$) or TCPTP$^{+/+}$ (EFM7$^{+/+}$) cells were serum starved and either left untreated or stimulated with 10 nM insulin for 60 min. Equal amounts of protein were resolved by SDS-PAGE and immunoblotted with antibodies specific for pTyr and then stripped and reprobed with antibodies specific for the IR β-subunit (IRβ). (C) EFM14$^{-/-}$ or EFM11$^{+/+}$ cells were serum starved and either left untreated or stimulated with 10 nM insulin for 60 min. Proteins were resolved and immunoblotted for pTyr and then stripped and reprobed for IR β-subunit. Molecular mass standards (prestained protein markers, broad range; New England Biolabs, Beverly, Mass.) are indicated on the right, and an arrow highlights the ≈95-kDa pTyr protein in TCPTP$^{-/-}$ cells following insulin stimulation. (D) TCPTP$^{-/-}$ (EFM4$^{-/-}$) or TCPTP$^{+/+}$ (EFM7$^{+/+}$) cells were serum starved and either left untreated or stimulated with 10 nM insulin for 60 min. Cells were lysed in immunoprecipitation lysis buffer, and the pTyr-containing proteins were immunoprecipitated as described under Materials and Methods. The immunoprecipitates (IPs) and equal quantities of the corresponding lysates prior to immunoprecipitation were resolved by SDS-PAGE and immunoblotted with antibodies specific for IRβ.

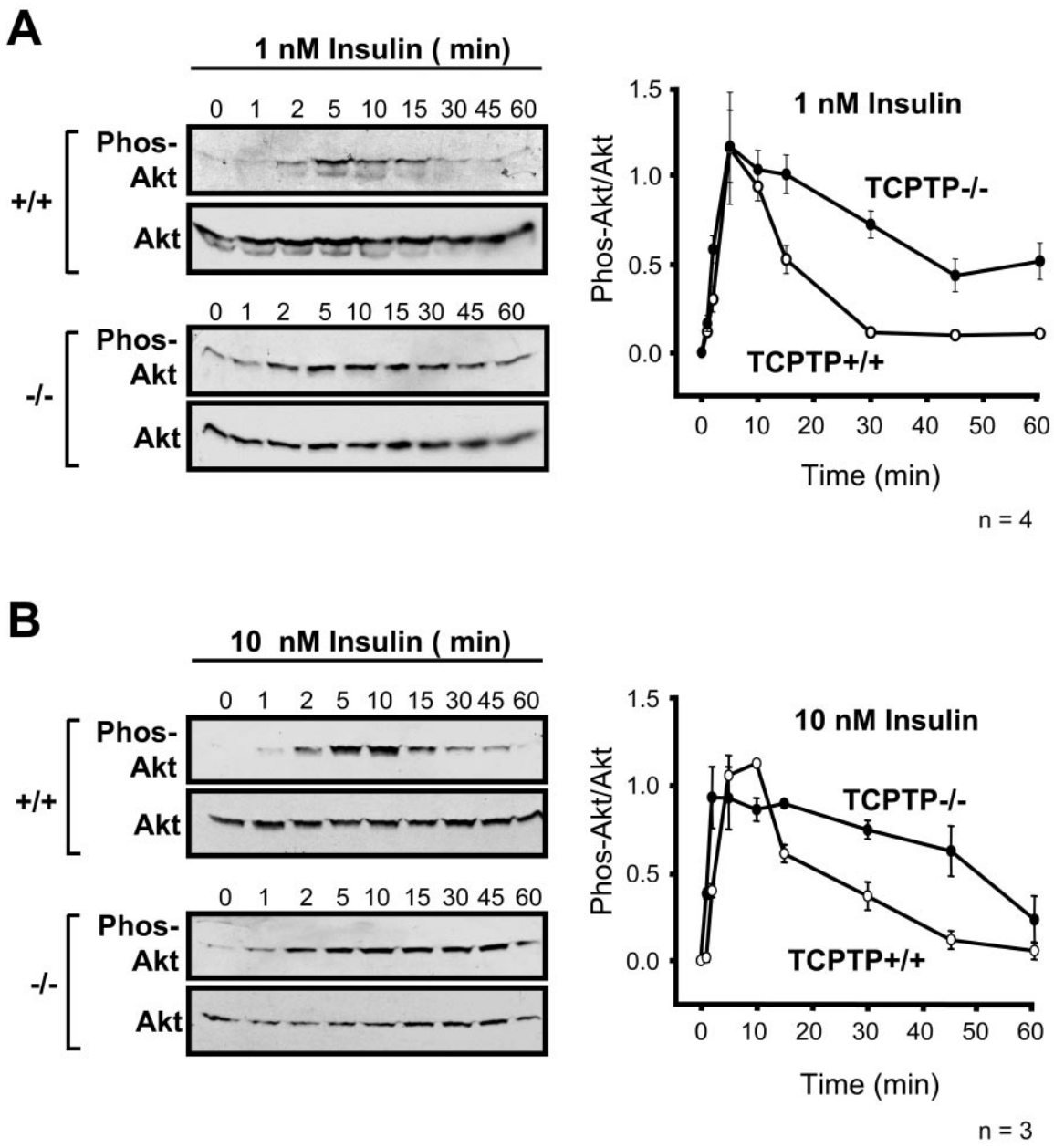

FIG. 5. Enhanced insulin-induced PKB/Akt signaling in TCPTP-null cells. (A) TCPTP$^{-/-}$ or TCPTP$^{+/+}$ cells were serum starved and either left untreated or stimulated with 1 nm (A) or 10 nm (B) insulin for 1 to 60 min. Cells were collected in hot 3× Laemmli sample buffer, and proteins were resolved by SDS-PAGE, transferred onto the same Immobilon-P (Millipore, Bedford, Mass.) membrane, and immunoblotted with antibodies specific for phosphorylated and activated PKB/Akt (Phos-Akt). Immunoblots were then stripped and reprobed with antibodies specific for total PKB/Akt protein. Representative immunoblots are shown. Quantitations of Akt phosphorylation in response to 1 nM or 10 nM insulin stimulation in TCPTP$^{-/-}$ and TCPTP$^{+/+}$ cells are also shown. Phospho-Akt immunoblots were quantitated by densitometric analysis and normalized for total Akt protein in corresponding Akt immunoblots, with the phospho-Akt/Akt ratio in the absence of insulin being set at zero. Units shown are arbitrary and are means ± standard errors of at least three independent experiments.

hanced insulin-induced IR β-subunit presence in pTyr immunoprecipitates from TCPTP$^{-/-}$ cells (Fig. 7A). These results indicate that reconstitution of physiologically relevant concentrations of TC45 can suppress the enhanced IR β-subunit tyrosine phosphorylation in TCPTP$^{-/-}$ cells.

We next examined whether TC45 reconstitution suppressed the enhanced insulin-induced PKB/Akt signaling observed in TCPTP$^{-/-}$ cells. Once more PKB/Akt activation was monitored with antibodies specific for the phosphorylated and activated PKB/Akt and normalized by densitometric analysis for total PKB/Akt protein. TC45 reconstitution suppressed the sustained PKB/Akt activity observed in TCPTP$^{-/-}$ cells in response to 1 nM insulin (Fig. 8A), so that PKB/Akt activation more closely resembled that in TCPTP$^{+/+}$ cells (Fig. 5A). At 10 nM insulin, TC45 reconstitution suppressed both the enhanced PKB/Akt activity that was observed in the TCPTP$^{-/-}$

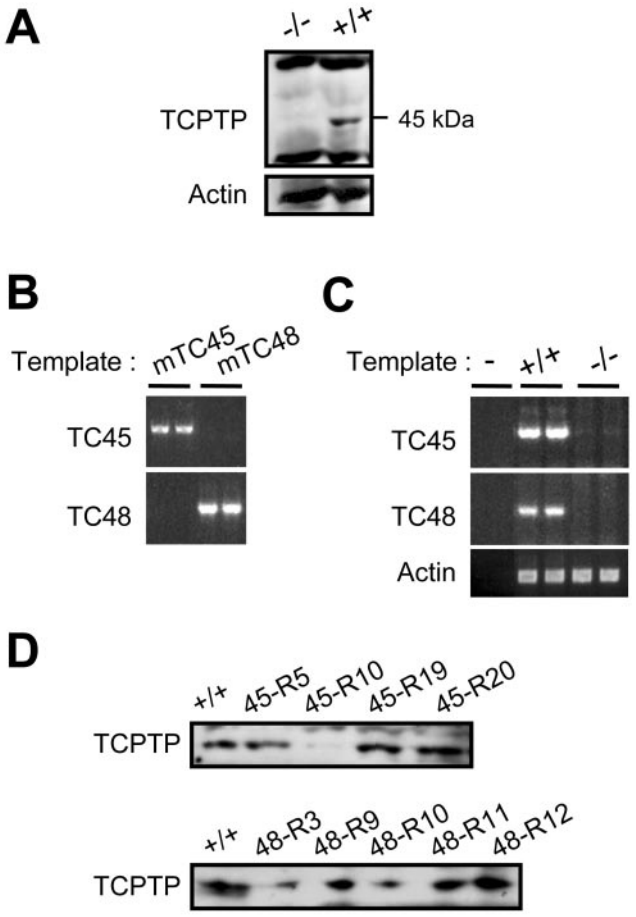

FIG. 6. TC48 and TC45 expression in TCPTP$^{+/+}$ cells. (A) Equal amounts of protein from TCPTP$^{-/-}$ (EFM4$^{-/-}$) and TCPTP$^{+/+}$ (EFM7$^{+/+}$) cells were resolved on SDS-PAGE and immunoblotted with polyclonal TCPTP 6228 antibodies. (B) PCR of purified murine TC48 (mTC48) or murine TC45 (mTC45) cDNA (1 pg of either mTC48 or mTC45 cDNA was used in a 25-μl reaction as indicated in Materials and Methods) with oligonucleotide primers specific for either TC45 or TC48 (indicated on the left). (C) Reverse transcription-PCR from TCPTP$^{+/+}$ or TCPTP$^{-/-}$ cells as indicated under Materials and Methods with oligonucleotide primers specific for either TC45 or TC48 (indicated on the left). Water was substituted for first-strand cDNA in negative controls (−), and β-actin reverse transcription-PCRs were used for normalization. PCRs in panels B and C were resolved on agarose gels and visualized by ethidium bromide staining. (D) Comparison of TCPTP expression in TCPTP$^{+/+}$ versus TC45-reconstituted (45-R5, 45-R10, 45-R19, and 45-R20) or TC48-expressing (48-R9, 48-R11, and 48-R12) cells. Equal amounts of protein from the indicated cell lines were resolved on SDS-PAGE and immunoblotted with polyclonal TCPTP 6228 antibodies.

relative to TCPTP$^{+/+}$ cells for the first 2 min of insulin stimulation and the sustained PKB/Akt activity observed at 15 to 45 min (Fig. 8B). These results demonstrate that the reconstitution of TC45 can suppress the enhanced/prolonged insulin-induced PKB/Akt signaling in TCPTP$^{-/-}$ cells so that it more closely resembles PKB/Akt signaling in TCPTP$^{+/+}$ cells. Therefore, the enhanced insulin-induced signaling in TCPTP$^{-/-}$ cells is attributable to the loss of TCPTP.

Although relatively insignificant levels of TC48 protein may be present in the TCPTP$^{+/+}$ cells, we could not exclude the possibility that TC48 might nevertheless contribute to IR regulation. As such, as a control we also determined whether TC48 could suppress the enhanced insulin-induced signaling in TCPTP$^{-/-}$ cells. As in the case of TC45 reconstitution, the expression of TC48 suppressed the phosphorylation of the ≈95-kDa pTyr protein that comigrated with the IR β-subunit and was detected in TCPTP$^{-/-}$ cells following insulin stimulation (Fig. 9A). Concordantly, TC48 expression suppressed the sustained activation of PKB/Akt in TCPTP$^{-/-}$ cells in response to 10 nM insulin (Fig. 9B). These results indicate that TC48 can also suppress the enhanced insulin-induced IR tyrosine phosphorylation and signaling in TCPTP$^{-/-}$ cells and therefore may also contribute to IR regulation in vivo.

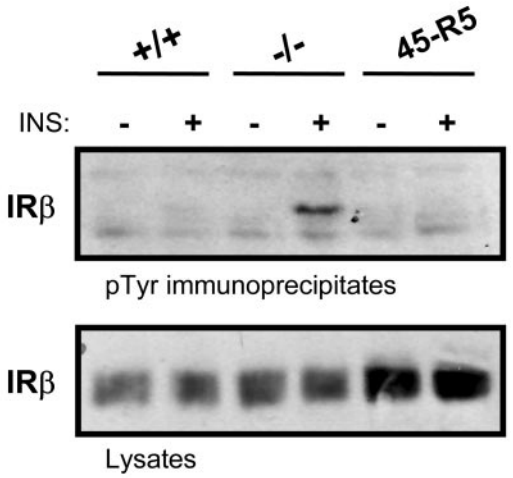

FIG. 7. TC45 reconstitution suppresses the enhanced IR β-subunit tyrosine phosphorylation in TCPTP-null cells. TCPTP$^{-/-}$, TCPTP$^{+/+}$, or 45-R5 cells were serum starved and either left untreated or stimulated with 10 nM insulin (INS) for 60 min. Cells were lysed, and the phosphotyrosine (pTyr)-containing proteins were immunoprecipitated as described under Materials and Methods. The immunoprecipitates and equal quantities of the corresponding lysates prior to immunoprecipitation were resolved by SDS-PAGE and immunoblotted with antibodies specific for IR β-subunit.

DISCUSSION

Although current attention has focused on the role of PTP1B in the regulation of the IR, data from PTP1B knockout mice (12, 28) indicate that more than one PTP acts to dephosphorylate and regulate the IR in vivo. Given the significant similarities in the three-dimensional structures of the TCPTP and PTP1B catalytic domains (25, 39), we speculated that the IR might be a physiological substrate of TCPTP. In this report, we demonstrate that TCPTP can recognize the IR as a cellular substrate and that IR activation and signaling are enhanced in cells that lack TCPTP but suppressed upon reconstitution of TCPTP. Taken together, our results provide persuasive evidence that TCPTP may regulate IR phosphorylation and signaling in vivo.

In this study we have shown that the otherwise nuclear TC45 can exit the nucleus and gain access to the IR in response to insulin stimulation. As in the case of the EGF receptor (46), the mechanism by which TC45 nuclear exit may occur is not clear; however, it may involve suppression of TC45 nuclear

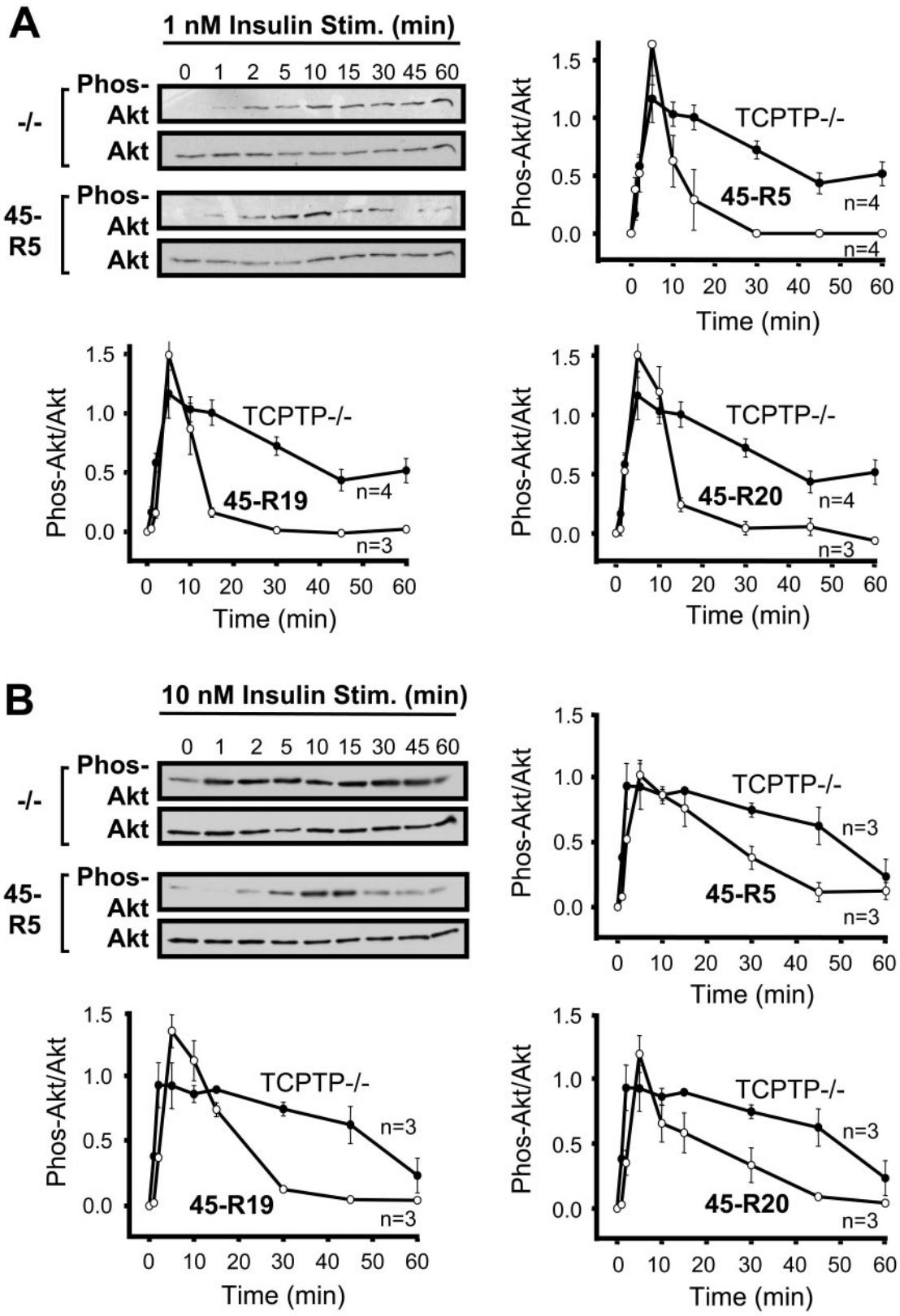

FIG. 8. TC45 reconstitution suppresses the enhanced insulin-induced PKB/Akt signaling in TCPTP-null cells. TCPTP$^{-/-}$ and either 45-R5, 45-R19, or 45-R20 cells were serum starved and either left untreated or stimulated with (A) 1 nM or (B) 10 nM insulin for 1 to 60 min. Cells were collected in hot 3× Laemmli sample buffer, and lysates were resolved by SDS-PAGE, transferred onto the same Immobilon-P membrane, and immunoblotted with antibodies specific for phosphorylated and activated PKB/Akt (Phos-Akt) and then stripped and reprobed for PKB/Akt.

import, as we have reported previously for the influence of cellular stress on TC45 localization (31). Nevertheless, with the possible exception of the STATs (2, 44), all other substrates identified to date for this "nuclear" phosphatase, including the EGF receptor, $p52^{Shc}$ (46, 48), the Janus-associated kinases (43), and now the IR, are in the cytoplasm.

One possibility is that the spatial isolation of TC45 in the nucleus may be essential for insulin-induced signal initiation, and TC45's nuclear exit may represent a negative feedback loop for the coordinated suppression of IR signaling. Such spatial isolation would also be pertinent to endoplasmic reticulum-localized phosphatases such as PTP1B and TC48. Presumably PTP1B and TC48 would only have access to the IR upon receptor endocytosis, and indeed this is the case for PTP1B and its recognition of the EGF receptor (13, 18). Notably, loss of PTP1B in cells has no significant impact on EGF receptor-mediated PKB/Akt signaling despite enhancing EGF receptor tyrosine phosphorylation (17). As such, EGF receptor-mediated PKB/Akt activation may occur predominantly at the plasma membrane. Consistent with this notion, we have shown previously that the overexpressed endoplasmic reticulum-localized TC48 does not suppress EGF receptor-mediated PKB/Akt activation, whereas TC45 does (48). In contrast, in the case of the IR, the effects of PTP1B loss on insulin-induced signaling in vivo (12, 28) and our data demonstrating that TC48 expression can suppress the enhanced insulin-induced PKB/Akt activation in TCPTP-null cells may indicate that IR-mediated PKB/Akt signaling occurs after endocytosis, whereupon TC48 and PTP1B would have ready access to the receptor. This is consistent with some previous studies indicating that a significant proportion of IR signaling occurs after endoyctosis and is associated with endosomes (27).

Despite our reverse transcription-PCR results indicating that TC48 message is present in the $TCPTP^{+/+}$ cells, by immunoblot analysis we could only detect TC45 protein. One possibility is that TC48 protein is present at low levels and its detection may be beyond the ability of our antibodies. Another possibility is that the electrophoretic mobilities of endogenous TC48 and TC45 in the $TCPTP^{+/+}$ cells overlap, possibly because of posttranslational modifications such as phosphorylation (19, 36). Irrespective, our data indicate that TC48 expression can complement the loss of TCPTP in TCPTP-null cells. Therefore, our studies demonstrate that both TCPTP variants have the capacity to regulate the IR and insulin-induced signaling in vivo. Although the $TCPTP^{+/+}$ cells may express principally TC45 and thus the enhanced IR activation and signaling in these murine cells may be attributable largely to the loss of TC45, it is plausible that in some circumstances, TC48 may be more abundant than TC45 and there, TC48 may have a more prominent role in IR regulation.

Previous studies utilizing the immortalized mouse embryo fibroblasts from TCPTP knockout animals indicated that PKB/Akt activation is not altered in response to platelet-derived growth factor (23), demonstrating that PKB/Akt signaling is not enhanced in general in the $TCPTP^{-/-}$ cells. We found that insulin-induced IR tyrosine phosphorylation and PKB/Akt signaling were enhanced in TCPTP-null cells and suppressed upon TCPTP reconstitution, providing persuasive evidence that TCPTP can regulate IR activation and signaling. Although our studies are consistent with TCPTP's acting on the IR, we cannot formally exclude the possibility that at least part of the effects of TCPTP on insulin-induced signaling in the TCPTP-null cells may be attributable to TCPTP's acting on substrates downstream of the IR that were not detected in our pTyr immunoblots. For example, previous studies have indicated that the phosphatase LAR may suppress insulin-induced signaling by dephosphorylating IRS-1 (59). In this study, we did not detect IRS-1 in TCPTP-D182A substrate-trapping mutant immunoprecipitates from 293 cells, nor did we detect a hyperphosphorylated protein corresponding to the molecular weight of IRS-1 in $TCPTP^{-/-}$ lysates, indicating that IRS-1 may not be a direct substrate of TCPTP.

The crystal structure of the PTP1B catalytic domain in complex with the Y1162/Y1163 tandemly phosphorylated IR β-subunit peptide revealed the existence of a second pTyr binding pocket in PTP1B that confers enhanced substrate binding (39). More recently, the crystal structure of the TCPTP catalytic domain indicated that this additional binding pocket also exists in TCPTP (25). Consistent with this, we had shown in early studies that TC45 recognizes $p52^{Shc}$ phosphorylated on Y239/Y240 in preference to Y317 (46). More recent data have indicated that both TCPTP and PTP1B recognize the doubly phosphorylated JAK protein tyrosine kinases (7, 35, 43). In this report we demonstrate that TCPTP can recognize the tandemly phosphorylated tyrosines 1162 and 1163 on the activation loop of the IR β-subunit and thus may suppress IR activation and signaling in a manner analogous to PTP1B. However, our studies do not preclude the possibility that TCPTP may recognize additional sites on the IR (20, 24, 37) or even that it may recognize such sites with greater efficiency.

Whereas TCPTP-null mice succumb to hematopoietic defects within weeks of birth (57), PTP1B-null mice develop normally but exhibit increased insulin sensitivity and obesity resistance (12, 28) attributable to IR (12) and JAK2 (7, 58) hyperphosphorylation, respectively. These differential phenotypes indicate that at least in mice, PTP1B and TCPTP are differentially required, with TCPTP being essential for normal hematopoiesis. The early lethality and the complexity associated with interpreting metabolic results from ailing TCPTP-null mice have prohibited an in vivo analysis of TCPTP's contribution to IR signaling. A number of possibilities exist as to how TCPTP and PTP1B may regulate the IR. They may dephosphorylate the IR in the same cell, or they may act in different cells or tissues. Our studies demonstrate that loss of TCPTP, even in the presence of PTP1B, enhances IR activation and signaling, indicating that the two phosphatases may act in concert in the same cell to regulate IR signaling in vivo.

Representative immunoblots are shown for clone 45-R5 in response to 1 or 10 nM insulin. Quantitations of PKB/Akt phosphorylation in response to insulin stimulation in $TCPTP^{-/-}$, 45-R5, 45-R19, and 45-R20 cells are also shown. In each case the phospho-Akt immunoblots were quantitated by densitometric analysis and normalized for total Akt protein in corresponding Akt immunoblots, with the phospho-Akt/Akt ratio in the absence of insulin being set at zero. Units are arbitrary and are means ± standard errors of at least three independent experiments.

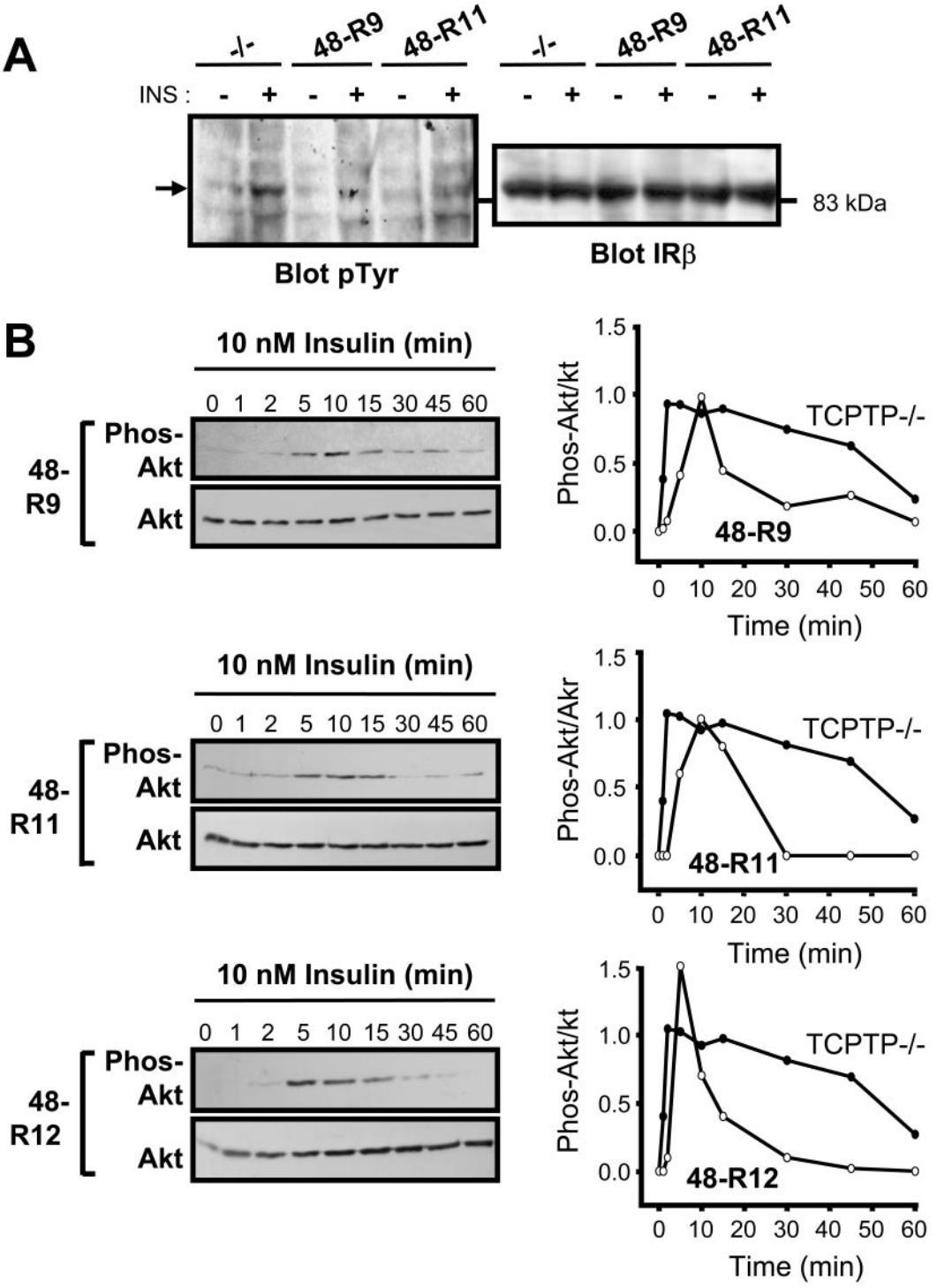

FIG. 9. TC48 expression suppresses enhanced insulin-induced signaling in TCPTP-null cells. (A) TCPTP$^{-/-}$ or TC48-expressing 48-R9 or 48-R11 cells were serum starved and either left untreated or stimulated with 10 nM insulin for 60 min. Equal amounts of protein were resolved by SDS-PAGE and immunoblotted with antibodies specific for phosphotyrosine (pTyr) and then stripped and reprobed with antibodies specific for the IR β-subunit (IRβ). IR β-subunit in the pTyr immunoblot is indicated by the arrow on the left. (B) 48-R9, 48-R11, or 48-R12 cells were serum starved and either left untreated or stimulated with 10 nM insulin for 1 to 60 min. Cells were collected in hot 3× Laemmli sample buffer, and lysates were resolved by SDS-PAGE and immunoblotted with antibodies specific for phosphorylated and activated PKB/Akt (Phos-Akt) and then stripped and reprobed for PKB/Akt. Representative immunoblots from two independent experiments for each of the TC48-reconstituted clones are shown. Also shown are the respective phospho-Akt/Akt quantitations relative to the kinetics of PKB/Akt phosphorylation in TCPTP$^{-/-}$ cells (as shown in Fig. 8, $n = 3$).

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S.G. and M.K.-H. contributed equally to this work.

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REFERENCES

1. **Andersen, J. N., O. H. Mortensen, G. H. Peters, P. G. Drake, L. F. Iversen, O. H. Olsen, P. G. Jansen, H. S. Andersen, N. K. Tonks, and N. P. Moller.** 2001. Structural and evolutionary relationships among protein tyrosine phosphatase domains. Mol. Cell. Biol. **21:**7117–7136.
2. **Aoki, N., and T. Matsuda.** 2002. A nuclear protein tyrosine phosphatase TC-PTP is a potential negative regulator of the PRL-mediated signaling pathway: dephosphorylation and deactivation of signal transducer and activator of transcription 5a and 5b by TC-PTP in nucleus. Mol. Endocrinol. **16:**58–69.
3. **Barford, D., A. J. Flint, and N. K. Tonks.** 1994. Crystal structure of human protein tyrosine phosphatase 1B. Science **263:**1397–1404.
4. **Barford, D., Z. Jia, and N. K. Tonks.** 1995. Protein tyrosine phosphatases take off. Nat. Struct. Biol. **2:**1043–1053.
5. **Bevan, P.** 2001. Insulin signalling. J. Cell Sci. **114:**1429–1430.
6. **Brown-Shimer, S., K. A. Johnson, J. B. Lawrence, C. Johnson, A. Bruskin, N. R. Green, and D. E. Hill.** 1990. Molecular cloning and chromosome mapping of the human gene encoding protein phosphotyrosyl phosphatase 1B. Proc. Natl. Acad. Sci. USA **87:**5148–5152.
7. **Cheng, A., N. Uetani, P. D. Simoncic, V. P. Chaubey, A. Lee-Loy, C. J. McGlade, B. P. Kennedy, and M. L. Tremblay.** 2002. Attenuation of leptin action and regulation of obesity by protein tyrosine phosphatase 1B. Dev. Cell **2:**497–503.
8. **Chernoff, J., A. R. Schievella, C. A. Jost, and R. L. Erikson.** 1990. Cloning of a cDNA for a major human protein-tyrosine-phosphatase. Proc. Natl. Acad. Sci. USA **87:**2735–2739.
9. **Cho, H., J. Mu, J. K. Kim, J. L. Thorvaldsen, Q. Chu, E. B. Crenshaw, 3rd, K. H. Kaestner, M. S. Bartolomei, G. I. Shulman, and M. J. Birnbaum.** 2001. Insulin resistance and a diabetes mellitus-like syndrome in mice lacking the protein kinase Akt2 (PKB beta). Science **292:**1728–1731.
10. **Drake, P. G., and B. I. Posner.** 1998. Insulin receptor-associated protein tyrosine phosphatase(s): role in insulin action. Mol. Cell. Biochem. **182:**79–89.
11. **Egawa, K., H. Maegawa, S. Shimizu, K. Morino, Y. Nishio, M. Bryer-Ash, A. T. Cheung, J. K. Kolls, R. Kikkawa, and A. Kashiwagi.** 2001. Protein tyrosine phosphatase-1B negatively regulates insulin signaling in L6 myocytes and Fao hepatoma cells. J. Biol. Chem. **276:**10207–10211.
12. **Elchebly, M., P. Payette, E. Michaliszyn, W. Cromlish, S. Collins, A. L. Loy, D. Normandin, A. Cheng, J. Himms-Hagen, C. C. Chan, C. Ramachandran, M. J. Gresser, M. L. Tremblay, and B. P. Kennedy.** 1999. Increased insulin sensitivity and obesity resistance in mice lacking the protein tyrosine phosphatase-1B gene. Science **283:**1544–1548.
13. **Flint, A. J., T. Tiganis, D. Barford, and N. K. Tonks.** 1997. Development of "substrate-trapping" mutants to identify physiological substrates of protein tyrosine phosphatases. Proc. Natl. Acad. Sci. USA **94:**1680–1685.
14. **Garton, A. J., A. J. Flint, and N. K. Tonks.** 1996. Identification of p130^{cas} as a substrate for the cytosolic protein tyrosine phosphatase PTP-PEST. Mol. Cell. Biol. **16:**6408–6418.
15. **Garton, A. J., and N. K. Tonks.** 1999. Regulation of fibroblast motility by the protein tyrosine phosphatase PTP-PEST. J. Biol. Chem. **274:**3811–3818.
16. **Goldstein, B. J., F. Ahmad, W. Ding, P. M. Li, and W. R. Zhang.** 1998. Regulation of the insulin signalling pathway by cellular protein-tyrosine phosphatases. Mol. Cell. Biochem. **182:**91–99.
17. **Haj, F. G., B. Markova, L. D. Klaman, F. D. Bohmer, and B. G. Neel.** 2003. Regulation of receptor tyrosine kinase signaling by protein tyrosine phosphatase-1B (PTP1B). J. Biol. Chem. **278:**739–744.
18. **Haj, F. G., P. J. Verveer, A. Squire, B. G. Neel, and P. I. Bastiaens.** 2002. Imaging sites of receptor dephosphorylation by PTP1B on the surface of the endoplasmic reticulum. Science **295:**1708–1711.
19. **Hao, L., T. Tiganis, N. K. Tonks, and H. Charbonneau.** 1997. The noncatalytic C-terminal segment of the T cell protein tyrosine phosphatase regulates activity via an intramolecular mechanism. J. Biol. Chem. **272:**29322–29329.
20. **He, W., T. J. O'Neill, and T. A. Gustafson.** 1995. Distinct modes of interaction of SHC and insulin receptor substrate-1 with the insulin receptor NPEY region via non-SH2 domains. J. Biol. Chem. **270:**23258–23262.
21. **Hill, M. M., S. F. Clark, D. F. Tucker, M. J. Birnbaum, D. E. James, and S. L. Macaulay.** 1999. A role for protein kinase Bbeta/Akt2 in insulin-stimulated GLUT4 translocation in adipocytes. Mol. Cell. Biol. **19:**7771–7781.
22. **Ibarra-Sanchez, M., P. D. Simoncic, F. R. Nestel, P. Duplay, W. S. Lapp, and M. L. Tremblay.** 2000. The T-cell protein tyrosine phosphatase. Semin. Immunol. **12:**379–386.
23. **Ibarra-Sanchez, M. J., J. Wagner, M. T. Ong, C. Lampron, and M. L. Tremblay.** 2001. Murine embryonic fibroblasts lacking TC-PTP display delayed G_1 phase through defective NF-κB activation. Oncogene **20:**4728–4739.
24. **Isakoff, S. J., Y. P. Yu, Y. C. Su, P. Blaikie, V. Yajnik, E. Rose, K. M. Weidner, M. Sachs, B. Margolis, and E. Y. Skolnik.** 1996. Interaction between the phosphotyrosine binding domain of Shc and the insulin receptor is required for Shc phosphorylation by insulin in vivo. J. Biol. Chem. **271:**3959–3962.
25. **Iversen, L. F., K. B. Moller, A. K. Pedersen, G. H. Peters, A. S. Petersen, H. S. Andersen, S. Branner, S. B. Mortensen, and N. P. Moller.** 2002. Structure determination of T cell protein tyrosine phosphatase. J. Biol. Chem. **20:**20.
26. **Jacob, K. K., J. Sap, and F. M. Stanley.** 1998. Receptor-like protein-tyrosine phosphatase alpha specifically inhibits insulin-increased prolactin gene expression. J. Biol. Chem. **273:**4800–4809.
27. **Kelly, K. L., and N. B. Ruderman.** 1993. Insulin-stimulated phosphatidylinositol 3-kinase. Association with a 185-kDa tyrosine-phosphorylated protein (IRS-1) and localization in a low density membrane vesicle. J. Biol. Chem. **268:**4391–4398.
28. **Klaman, L. D., O. Boss, O. D. Peroni, J. K. Kim, J. L. Martino, J. M. Zabolotny, N. Moghal, M. Lubkin, Y. B. Kim, A. H. Sharpe, A. Stricker-Krongrad, G. I. Shulman, B. G. Neel, and B. B. Kahn.** 2000. Increased energy expenditure, decreased adiposity, and tissue-specific insulin sensitivity in protein-tyrosine phosphatase 1B-deficient mice. Mol. Cell. Biol. **20:**5479–5489.
29. **Klingler-Hoffmann, M., M. T. Fodero-Tavoletti, K. Mishima, Y. Narita, W. K. Cavenee, F. B. Furnari, H. J. Huang, and T. Tiganis.** 2001. The protein tyrosine phosphatase TCPTP suppresses the tumorigenicity of glioblastoma cells expressing a mutant EGF receptor. J. Biol. Chem. **276:**46313–46318.
30. **Krueger, N. X., M. Streuli, and H. Saito.** 1990. Structural diversity and evolution of human receptor-like protein tyrosine phosphatases. EMBO J. **9:**3241–3252.
31. **Lam, M. H., B. J. Michell, M. T. Fodero-Tavoletti, B. E. Kemp, N. K. Tonks, and T. Tiganis.** 2001. Cellular stress regulates the nucleocytoplasmic distribution of the protein tyrosine phosphatase TCPTP. J. Biol. Chem. **276:**37700–37707.
32. **Lammers, R., B. Bossenmaier, D. E. Cool, N. K. Tonks, J. Schlessinger, E. H. Fischer, and A. Ullrich.** 1993. Differential activities of protein tyrosine phosphatases in intact cells. J. Biol. Chem. **268:**22456–22462.
33. **Lorenzen, J. A., C. Y. Dadabay, and E. H. Fischer.** 1995. COOH-terminal sequence motifs target the T-cell protein tyrosine phosphatase to the endoplasmic reticulum and nucleus. J. Cell Biol. **131:**631–643.
34. **Matthews, R. J., E. D. Cahir, and M. L. Thomas.** 1990. Identification of an additional member of the protein-tyrosine-phosphatase family: evidence for alternative splicing in the tyrosine phosphatase domain. Proc. Natl. Acad. Sci. USA **87:**4444–4448.
35. **Myers, M. P., J. N. Andersen, A. Cheng, M. L. Tremblay, C. M. Horvath, J. P. Parisien, A. Salmeen, D. Barford, and N. K. Tonks.** 2001. TYK2 and JAK2 are substrates of protein-tyrosine phosphatase 1B. J. Biol. Chem. **276:**47771–47774.
36. **Nambirajan, S., V. Radha, S. Kamatkar, and G. Swarup.** 2000. PTP-S2, a nuclear tyrosine phosphatase, is phosphorylated and excluded from condensed chromosomes during mitosis. J. Biosci. **25:**33–40.
37. **Paz, K., S. Boura-Halfon, L. S. Wyatt, D. LeRoith, and Y. Zick.** 2000. The juxtamembrane but not the carboxyl-terminal domain of the insulin receptor mediates insulin's metabolic functions in primary adipocytes and cultured hepatoma cells. J. Mol. Endocrinol. **24:**419–432.
38. **Ren, J. M., P. M. Li, W. R. Zhang, L. J. Sweet, G. Cline, G. I. Shulman, J. N. Livingston, and B. J. Goldstein.** 1998. Transgenic mice deficient in the LAR protein-tyrosine phosphatase exhibit profound defects in glucose homeostasis. Diabetes **47:**493–497.
39. **Salmeen, A., J. N. Andersen, M. P. Myers, N. K. Tonks, and D. Barford.** 2000. Molecular basis for recognition and dephosphorylation of the activation segment of the insulin receptor by protein tyrosine phosphatase 1B. Mol. Cell **6:**1401–1412.
40. **Saltiel, A. R., and C. R. Kahn.** 2001. Insulin signalling and the regulation of glucose and lipid metabolism. Nature **414:**799–806.
41. **Sap, J., P. D'eustachio, and J. Schlessinger.** 1990. Cloning and expression of a widely expressed receptor tyrosine phosphatase. Proc. Natl. Acad. Sci. USA **87:**6112–6116.
42. **Schaapveld, R. Q., J. T. Schepens, G. W. Robinson, J. Attema, F. T. Oerlemans, J. A. Fransen, M. Streuli, B. Wieringa, L. Hennighausen, and W. J. Hendriks.** 1997. Impaired mammary gland development and function in mice lacking LAR receptor-like tyrosine phosphatase activity. Dev. Biol. **188:**134–146.
43. **Simoncic, P. D., A. Lee-Loy, D. L. Barber, M. L. Tremblay, and C. J. McGlade.** 2002. The T cell protein tyrosine phosphatase is a negative regulator of janus family kinases 1 and 3. Curr. Biol. **12:**446–453.
44. **ten Hoeve, J., M. J. Ibarra-Sanchez, Y. Fu, W. Zhu, M. Tremblay, M. David,**

and K. Shuai. 2002. Identification of a nuclear Stat1 protein tyrosine phosphatase. Mol. Cell. Biol. **22:**5662–5668.
45. **Tiganis, T.** 2002. Protein tyrosine phosphatases: dephosphorylating the EGF receptor. IUBMB Life **53:**3–14.
46. **Tiganis, T., A. M. Bennett, K. S. Ravichandran, and N. K. Tonks.** 1998. Epidermal growth factor receptor and the adaptor protein $p52^{Shc}$ are specific substrates of T-cell protein tyrosine phosphatase. Mol. Cell. Biol. **18:**1622–1634.
47. **Tiganis, T., A. J. Flint, S. A. Adam, and N. K. Tonks.** 1997. Association of the T-cell protein tyrosine phosphatase with nuclear import factor p97. J. Biol. Chem. **272:**21548–21557.
48. **Tiganis, T., B. E. Kemp, and N. K. Tonks.** 1999. The protein tyrosine phosphatase TCPTP regulates EGF receptor-mediated and phosphatidylinositol 3-kinase-dependent signalling. J. Biol. Chem. **274:**27768–27775.
49. **Tonks, N. K., C. D. Diltz, and E. H. Fischer.** 1988. Characterization of the major protein-tyrosine-phosphatases of human placenta. J. Biol. Chem. **263:**6731–6737.
50. **Tonks, N. K., C. D. Diltz, and E. H. Fischer.** 1988. Purification of the major protein-tyrosine-phosphatases of human placenta. J. Biol. Chem. **263:**6722–6730.
51. **Tonks, N. K., and B. G. Neel.** 2001. Combinatorial control of the specificity of protein tyrosine phosphatases. Curr. Opin. Cell Biol. **13:**182–195.
52. **Venable, C. L., E. U. Frevert, Y. B. Kim, B. M. Fischer, S. Kamatkar, B. G. Neel, and B. B. Kahn.** 2000. Overexpression of protein-tyrosine phosphatase-1B in adipocytes inhibits insulin-stimulated phosphoinositide 3-kinase activity without altering glucose transport or Akt/protein kinase B activation. J. Biol. Chem. **275:**18318–18326.
53. **Walchli, S., M. L. Curchod, R. P. Gobert, S. Arkinstall, and R. Hooft van Huijsduijnen.** 2000. Identification of tyrosine phosphatases that dephosphorylate the insulin receptor. A brute force approach based on "substrate-trapping" mutants. J. Biol. Chem. **275:**9792–9796.
54. **White, M. F.** 1997. The insulin signalling system and the IRS proteins. Diabetologia **40**(Suppl. 2)**:**S2–S17.
55. **White, M. F.** 1998. The IRS-signalling system: a network of docking proteins that mediate insulin action. Mol. Cell. Biochem. **182:**3–11.
56. **Yamauchi, K., V. Ribon, A. R. Saltiel, and J. E. Pessin.** 1995. Identification of the major SHPTP2-binding protein that is tyrosine-phosphorylated in response to insulin. J. Biol. Chem. **270:**17716–17722.
57. **You-Ten, K. E., E. S. Muise, A. Itie, E. Michaliszyn, J. Wagner, S. Jothy, W. S. Lapp, and M. L. Tremblay.** 1997. Impaired bone marrow microenvironment and immune function in T cell protein tyrosine phosphatase-deficient mice. J. Exp. Med. **186:**683–693.
58. **Zabolotny, J. M., K. K. Bence-Hanulec, A. Stricker-Krongrad, F. Haj, Y. Wang, Y. Minokoshi, Y. B. Kim, J. K. Elmquist, L. A. Tartaglia, B. B. Kahn, and B. G. Neel.** 2002. PTP1B regulates leptin signal transduction in vivo. Dev. Cell **2:**489–495.
59. **Zabolotny, J. M., Y. B. Kim, O. D. Peroni, J. K. Kim, M. A. Pani, O. Boss, L. D. Klaman, S. Kamatkar, G. I. Shulman, B. B. Kahn, and B. G. Neel.** 2001. Overexpression of the LAR (leukocyte antigen-related) protein-tyrosine phosphatase in muscle causes insulin resistance. Proc. Natl. Acad. Sci. USA **98:**5187–5192.
60. **Zhu, W., T. Mustelin, and M. David.** 2002. Arginine methylation of STAT1 regulates its dephosphorylation by T cell protein tyrosine phosphatase. J. Biol. Chem. **277:**35787–35790.