

TPC1 Knockout Knocks Out TPC1

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Two-pore channels (TPCs) are ancient ion channels that reside within acidic organelles, such as endosomes and lysosomes (1). TPCs are the proposed target of the second messenger nicotinic acid adenine dinucleotide phosphate (NAADP), which mobilizes Ca^{2+} from “acidic Ca^{2+} stores” (2). However, fundamental aspects of TPC biology—how they activate and their native ionic permeability (3–8)—remain controversial (reviewed in references 9 and 10).

Photolabeling studies using a radioactive NAADP-derived probe (5N_3 -[^{32}P]NAADP) have suggested that NAADP may not directly bind to TPCs but may stimulate channel activity via small NAADP-binding proteins (~23 kDa) associated with the TPC complex (11–14). One piece of evidence supporting this idea is the preservation of photolabeling in TPC knockout mice (11). For example, as shown in Fig. 1A, NAADP-specific labeling at 23 kDa is apparent in the knockout $\text{TPC1}^{-/-}$ mouse sample and selectively displaced by unlabeled NAADP, with affinity similar to that of wild-type mice. These data indicate that TPCs are unlikely the direct targets of NAADP.

In a recently published study (15), Ruas and colleagues challenge this interpretation. Their data suggest the existence of a mouse TPC1 variant (TPC1B) with a truncated NH_2 -terminal domain compared to the previously studied TPC1 isoform (TPC1A). If this TPC1B variant were to be prevalently expressed, or if gene trap skipping occurred in the previously used *Tpcn1* null line (*Tpcn1*^{LEXKO-471}) to preserve TPC1A expression, then for either of these reasons, samples would not be TPC1 null. This would invalidate the conclusion of prior photolabeling experiments (11). We concur that it is important to investigate TPC1 expression to confirm the status of this mouse line as TPC1 null.

In this regard, TPC1B represents an NH_2 -terminal truncation of 69 residues relative to TPC1A (Fig. 1B). While no evidence demonstrating endogenous expression of TPC1B protein or the abundance of the TPC1B variant relative to wild-type TPC1A was provided, we note that a similarly truncated construct (85 residues) has previously been shown by us to be retained in the endoplasmic reticulum (ER) (16). Indeed, ER retention of TPC1B is increased in colocalization data presented by Ruas and colleagues (see Fig. 4D in reference 15). Further, the blot of overexpressed TPC1B (Fig. 4B in reference 15) shows evidence of impaired glycosylation (important for TPC function [17]), suggesting that TPC1B may be poorly expressed.

To assess TPC1 expression in the *Tpcn1*^{LEXKO-471} mouse model used previously in our photolabeling experiments (11), Western blotting was performed with a validated antibody (16, 18) capable of recognizing a COOH-terminal epitope present in both TPC1 isoforms (Fig. 1B). The proposed TPC1B truncation would be similar in size to full-length TPC1A (predicted at 87 versus 94 kDa for nascent forms). Our data revealed a complete loss of TPC1 immunoreactivity at this size (Fig. 1C and D). This result under-

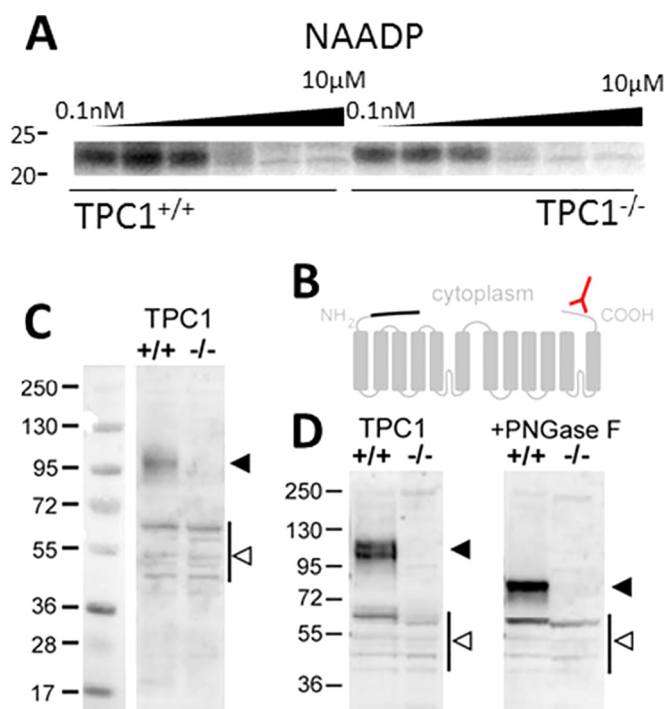


FIG 1 TPC1 protein is absent in transgenic TPC1 mice. (A) Photolabeling data using 5N_3 -[^{32}P]NAADP showing specific photolabeling of an ~23-kDa candidate NAADP binding protein in mouse pancreatic samples from a matched littermate mouse ($\text{TPC1}^{+/+}$) [left] and a TPC1 knockout mouse ($\text{TPC1}^{-/-}$) [right]; *Tpcn1*^{LEXKO-471}. Further detail is available in reference 11. (B) Schematic of TPC1 membrane topology to highlight the COOH-terminal epitope (red) present in wild-type TPC1 and the putative NH_2 -terminal truncation of TPC1B (the missing sequence is shown with a black line). (C and D) Western blot analysis of liver samples from wild-type ($+/+$) and *Tpcn1*^{LEXKO-471} ($-/-$) mice described in reference 11 using an antibody raised to the C terminus of TPC1 (ab80961; Abcam). (D) Samples were treated with peptide-N-glycosidase F (PNGase F) (right) to remove N-linked oligosaccharides as described in reference 17. Note the absence of major immunoreactive bands (solid arrowheads) in the transgenic mice. Bands present in both samples (open arrowheads) are likely nonspecific, but we cannot rule out the possibility of severely truncated, nonfunctional TPC1 variants that escape inactivation. Numbers at the left of the gels are molecular masses (in kilodaltons).

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scores prior conclusions (11–13). Repetition of our published photolabeling experiments with *Tpcn1*^{T159} samples characterized by Ruas and colleagues (15) is expected to yield a similar result. Comparison of whole animal phenotypes between different *TPC1*^{-/-} strains will require further investigation.

In conclusion, the *Tpcn1*^{LEXKO-471} mouse line can be employed by the community as a *Tpcn1*^{-/-} sample.

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