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Reply to "TPC1 Knockout Knocks Out TPC1"

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In a recent study (1), the pattern and intensity of proteins labeled with a photoaffinity probe ($5N_3$-[^{32}P]NAADP) were unchanged between samples from wild-type (WT) *Tpcn1*$^{\text{LEXKO-471}}$ mice, from which the authors concluded that TPC1 was not an NAADP-binding protein. However, no data were provided to assess the status of TPC1 expression in the *Tpcn1*$^{\text{LEXKO-471}}$ mutant mice.

In our recent study on a different *Tpcn1* mutant mouse line (*Tpcn1*$^{\text{T159}}$) (generated by a gene trap insertion in the same *Tpcn1* intron and in very close proximity to the insertion site of the gene trap in *Tpcn1*$^{\text{LEXKO-471}}$), we provided evidence that this gene trap locus did not significantly affect the expression of either *Tpcn1A* or *Tpcn1B* transcripts (2). Consequently, we expressed our concerns regarding the suitability of the similar *Tpcn1*$^{\text{LEXKO-471}}$ mice as a valid TPC1 knockout (KO) model and highlighting the need for a thorough characterization of the *Tpcn1*$^{\text{LEXKO-471}}$ line.

In their letter, Hooper et al. (3) now provide some data related to the status of *Tpcn1* expression in *Tpcn1*$^{\text{LEXKO-471}}$ mice. Their immunoblot images show a significant reduction in the intensity of the immunoreactive band believed to correspond to TPC1A, and this therefore is good evidence that the *Tpcn1*$^{\text{LEXKO-471}}$ mutation has an impact on TPC1 expression.

However, with immunoblotting, one cannot rule out the possibility of residual expression of TPC1A or TPC1B, which might be unmasked by higher exposure and/or higher antibody concentration. Therefore, although the data presented by the authors go some way to characterize the *Tpcn1* expression status in *Tpcn1*$^{\text{LEXKO-471}}$ mice, additional, more sensitive and definitive assessments of expression (e.g., reverse transcription-PCR data showing the lack of *Tpcn1A* and *Tpcn1B* transcripts) as well as functional assessments will be important to fully establish whether *Tpcn1*$^{\text{LEXKO-471}}$ is a complete TPC1 KO rather than a TPC1 hypomorphic model.

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